Probing Host-Selective Phytotoxicity: Synthesis and Biological Activity of Phomalide, Isophomalide, and Dihydrophomalide

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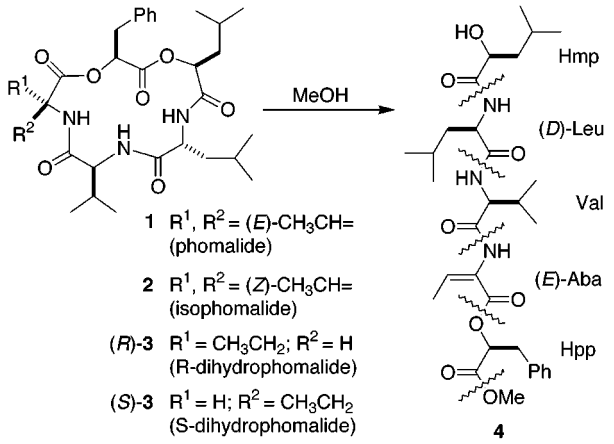
The cyclic depsipeptide phomalide [cyclo(Val-(*E*)-Aba-Hpp-Hmp-(*R*)-Leu); Aba = 2-amino-2-butenoic acid, Hpp = (2*S*)-2-hydroxy-3-phenylpropanoic acid, Hmp = (2*S*)-2-hydroxy-4-methylpentanoic acid] is the host-selective phytotoxin produced by the fungus [*Leptosphaeria maculans* (Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] which causes blackleg disease (a devastating disease of several economically important brassica crops). Efficient total syntheses of phomalide, its (*Z*)-isomer isophomalide, and the two dihydro analogues [(*R*)-dihydrophomalide and (*S*)-dihydrophomalide] are described. A [2 + 3] fragment coupling of Cbz-Val-(*Z*)-Aba with Hpp-Hmp-D-Leu-OBn followed by deprotection and cyclization gave isophomalide which was diastereoselectively isomerized to phomalide by conjugate addition of PhSeH followed by elimination of the corresponding selenoxide. The dihydro analogues were prepared similarly using Cbz-Val-(*R*)-Abu or Cbz-Val-(*S*)-Abu (Abu = 2-aminobutanoic acid) in place of Cbz-Val-(*Z*)-Aba. Biological evaluations of phomalide, isophomalide, and the dihydrophomalides revealed that only phomalide (10^{-5} M) caused necrotic, chlorotic, and reddish lesions on canola (*Brassica napus* and *Brassica rapa*; susceptible to blackleg) leaves whereas no damage was observed on brown mustard (*Brassica juncea*; resistant to blackleg) or white mustard (*Sinapis alba*; resistant to blackleg) leaves, even at significantly higher concentrations (10^{-4} M). Thus, both the presence and configuration of the double bond is crucial for selective phytotoxicity. This is the first reported synthesis of an (*E*)-Aba-containing natural product, and importantly, the (*Z*) → (*E*) isomerization approach should be applicable to other (depsi)peptide targets thereby allowing investigation of the effect of the double-bond configuration on various properties.

Introduction

The ability of a fungal pathogen to damage host plant cells can involve the biosynthesis and release of host-selective phytotoxins.[1] Host-selective phytotoxins are chemical signals which facilitate pathogen penetration and colonization of host plant tissues but do not significantly affect non host plants. Although many fungal diseases appear to be mediated by host-selective toxins, the molecular basis for the selectivity of this process is not well understood in the majority of the cases.[2] One such disease, blackleg, affects several economically important brassica crops and can be particularly devastating for the oilseeds canola (*Brassica napus* and *Brassica rapa*) and rapeseed (*B. napus* and *B. rapa*).[3] Recently, a better understanding of the blackleg causing fungus [*Leptosphaeria maculans* (Desm.) Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] was achieved with the isolation of phomalide (**1**), a host-selective toxin that appears to be involved in disease development.[4]

Phomalide (**1**) is a rather unusual cyclic depsipeptide composed of three α-amino acid and two α-hydroxy acid residues. The identity of the individual residues and their sequence were deduced from spectroscopic data.[4] The fragmentation pattern in the mass spectrum of the



derived methyl ester **4** served to corroborate the sequence suggested by NMR (HMBC). The absolute configurations of the L-valine (Val), D-leucine (D-Leu), (2*S*)-2-hydroxy-4-methylpentanoic acid (Hmp), and (2*S*)-2-hydroxy-3-phenylpropanoic acid (Hpp) residues were firmly established by acid hydrolysis of **1** and comparison of the resulting intact residues with authentic samples.[4,5] The (*E*)-configuration of the 2-amino-2-butenoic (Aba) residue in **1** was assigned on the basis of the observation of a positive NOE for the vinylic proton upon saturation of the enamine N−H. Within this class of natural products,[6] phomalide (**1**) has several noteworthy structural features including the 15-membered ring, the consecutive ester

(1) For a recent multiauthor review on phytotoxins, see: Graniti, A. et al. *Experientia* **1991**, *47*, 751−826.
(2) Kohomoto, K.; Otani, H. *Experientia* **1991**, *47*, 756−764.
(3) For a recent review on the history and control of blackleg disease, see: Gugel, R. K.; Petrie, G. A. *Can. J. Plant Pathol.* **1992**, *14*, 36−45.
(4) Pedras, M. S. C.; Taylor, J. T.; Nakashima, T. T. *J. Org. Chem.* **1993**, *58*, 4778−4780.
(5) Pedras, M. S. C. *Can. J. Chem.* **1997**, *75*, 314−317.

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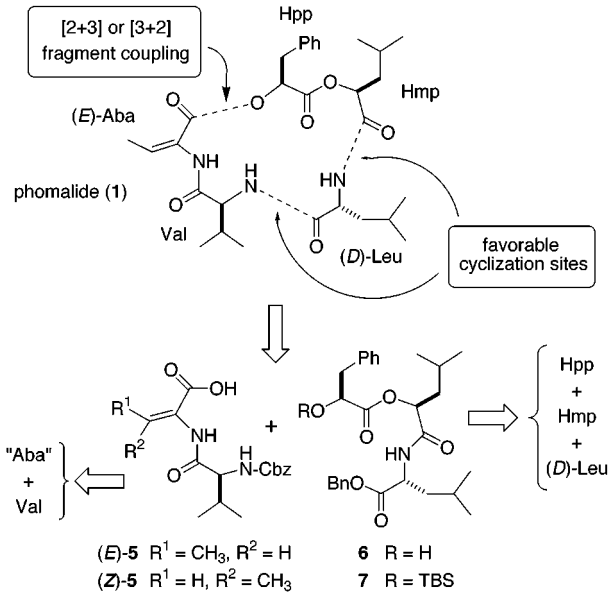
linkages, and particularly the unusual (*E*)-Aba residue. Numerous peptides and depsipeptides that incorporate α,β-unsaturated amino acid residues and possess diverse biological activity have been reported;[7,8] where diastereoisomerism is possible, those residues are most often present in the thermodynamically more stable (*Z*)-configuration.

Because of its host-selective phytotoxicity, phomalide (**1**) is an excellent probe for investigating the blackleg disease resistance of agriculturally important *Brassica* species. However, due to significant problems associated with obtaining sufficient quantities of **1** from fungal cultures,[9,10] its chemical synthesis became an essential prerequisite for biological studies. The synthetic strategy typically employed for the preparation of cyclic depsipeptides (and peptides) involves linear or convergent coupling of intact hydroxy acid and amino acid fragments followed by cyclization.[11] This approach reduces the key strategic decisions to the site of cyclization and the order of the residue coupling. Application of this strategy to phomalide (**1**) leaves the introduction of the Aba residue and control of its stereochemistry as major concerns. Typically, α,β-unsaturated amino acid residues show reduced reactivity in both *C*- and *N*-terminal acylations and the free amino forms hydrolyze readily.[7,12] As a consequence, α,β-unsaturated amino acids are usually not incorporated into peptides as intact residues but rather are elaborated from suitable precursor residues after peptide coupling and often late in the synthesis. Most syntheses of Aba derivatives give the thermodynamically more stable (*Z*)-isomers selectively[7] and stereoselective syntheses of (*Z*)-Aba-containing natural products by incorporating threonine as the surrogate residue with subsequent β-elimination have been described.[13] Although the stereoselective preparation of simple (*E*)-Aba derivatives has been achieved by syn-elimination of certain threonine derivatives[14] or by anti-elimination of *allo*-threonine derivatives,[15] these methods have not been applied to stereocontrolled peptide (or depsipeptide) synthesis.

Scheme 1



It is generally recognized that the presence of α,β-unsaturated amino acid residues imparts unique reactivity and conformational properties to peptides. Nonetheless, in part due to the lack of access to the (*E*)-isomers, the effect of the double-bond configuration on these properties has not been investigated.[7] In fact, to the best of our knowledge, the synthesis of an (*E*)-Aba-containing natural product has not been previously reported. In this paper we report the stereoselective syntheses of phomalide (**1**), its (*Z*)-isomer isophomalide (**2**), and the two dihydro analogues (*R*)-dihydrophomalide [(*R*)-**3**] and (*S*)-dihydrophomalide [(*S*)-**3**].[16] In addition, we establish that the double-bond configuration has a remarkable effect on the toxicity of these molecules to blackleg resistant and susceptible plants.

Results and Discussion

Our retrosynthetic analysis of phomalide (**1**) is presented in Scheme 1. The choice of the cyclization site is often crucial to the successful synthesis of a cyclic peptide or depsipeptide and a number of guidelines for effective selection have evolved.[11c,12,17] Consideration of these ideas focused our attention on the D-Leu-Val and Hmp-D-Leu

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(7) For reviews, see: (a) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159−172. (b) Noda, K.; Shimohigashi, Y.; Izumiya, N. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1983; Vol. 5, pp 285−339.

(8) For some recent examples of peptides and depsipeptides containing the (*Z*)-Aba residue, see: (a) Bonnard, I.; Rolland, M.; Francisco, C.; Banaigs, B. *Lett. Pept. Sci*. **1997**, *4*, 289−292. (b) Hamann, M. T.; Otto, C. S.; Scheuer, P. J.; Dunbar, D. C. *J. Org. Chem*. **1996**, *61*, 6594−6600. (c) Ballio, A.; Bossa, F.; Camoni, L.; Di Giorgio, D.; Flamand, M.-C.; Maraite, H.; Nitti, G.; Pucci, P.; Scaloni, A. *FEBS Lett*. **1996**, *381*, 213-216. (d) Namikoshi, M.; Choi, B. W.; Sakai, R.; Sun, F.; Carmichael, W. W.; Evans, W. R.; Cruz, P. *J. Org. Chem*. **1994**, *59*, 2349−2357. (e) Gulavita, N. K.; Pomponi, S. A.; Wright, A. E.; Yarwood, D.; Sills, M. A. *Tetrahedron Lett*. **1994**, *35*, 6815−1688. (f) Ballio, A.; Bossa, F.; Di Giorgio, D.; Ferranti, P.; Paci, M.; Pucci, P.; Scaloni, A.; Segre, A.; Strobel, G. A. *FEBS Lett*. **1994**, *355*, 96−100. (g) Shigematsu, N.; Ueda, H.; Takase, S.; Tanaka, H.; Yamamoto, K.; Tada, T. *J. Antibiot*. **1994**, *47*, 311−314. (h) Scaloni, A.; Bachmann, R. C.; Takemoto, J. Y.; Barra, D.; Simmaco, M.; Ballio, A. *Nat. Prod. Lett*. **1994**, *4*, 159−164. For examples containing an (*E*)-Aba residue, see: (i) Sano, T.; Kaya, K. *Tetrahedron* **1998**, *54*, 463−870. (j) Schummer, D.; Forche, E.; Wray, V.; Domke, T.; Reichenbach, H.; Hoefle, G. *Liebigs Ann*. **1996**, 971−978. (k) Bewley, C. A.; He, H.; Williams, D. H.; Faulkner, D. J. *J. Am. Chem. Soc*. **1996**, *118*, 4314−4321. (l) Pergament, I.; Carmeli, S. *Tetrahedron Lett*. **1994**, *35*, 8473−8476. (m) Minami, Y.; Yoshida, K.-i.; Azuma, R.; Urakawa, A.; Kawauchi, T.; Otani, T. *Tetrahedron Lett*. **1994**, *35*, 8001−8004.

(9) For a recent review on phytotoxins of the blackleg fungus, see: Pedras, M. S. C. *Rev. Latinoam. Quim*. **1996**, *24*, 177−182.

(10) Pedras, M. S. C.; Bisenthal, C. J. *Can. J. Microbiol*. **1998**, *44*, 547−553

(11) (a) Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed.; Springer-Verlag: Berlin, 1993. (b) Izumiya, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. *Synthetic Aspects of Biologically Acitve Cyclic Peptides*; Halsted Press: New York, 1979. (c) Kopple, K. D. *J. Pharm. Sci*. **1972**, *61*, 1345−1356.

(12) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev*. **1997**, *97*, 2243−2266.

(13) (a) Li, K. W.; Wu, J.; Xing, W.; Simon, J. A. *J. Am. Chem. Soc*. **1996**, *118*, 7237−7238. (b) Valentekovich, R. J.; Schreiber, S. L. *J. Am. Chem. Soc*. **1995**, *117*, 9069−9070. (c) Meldal, M.; Bielfeldt, T.; Peters, S.; Jensen, K. J.; Paulsen, H.; Bock, K. *Int. J. Pept. Protein Res*. **1994**, *43*, 529−536. (d) Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn*. **1992**, *65*, 2227−2240. (e) Schmidt, U.; Mundinger, K.; Mangold, R.; Lieberknecht, A. *J. Chem. Soc., Chem. Commun*. **1990**, 1216−1219. (f) Mihara, H.; Aoyagi, H.; Ueno, T.; Kato, T.; Izumiya, N. *Bull. Chem. Soc. Jpn*. **1986**, *59*, 2041−2043.

(14) Rich, D. H.; Tam, J. P. *J. Org. Chem*. **1977**, *42*, 3815−3820, and cited references.

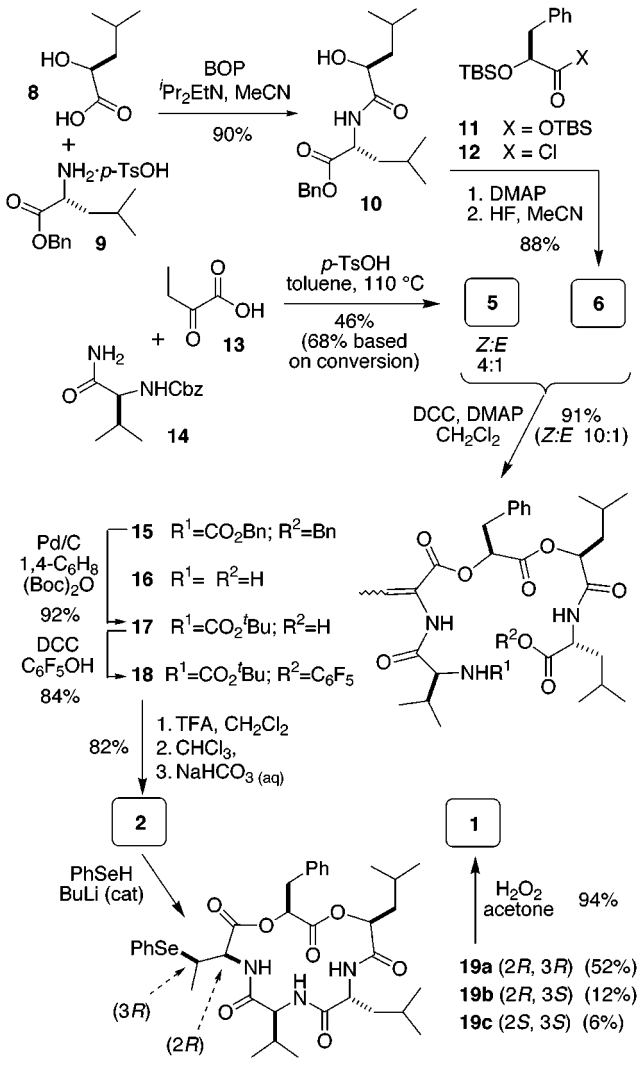
(15) Somekh, L.; Shanzer, A. *J. Org. Chem*. **1983**, *48*, 907−908, and cited references.

(16) For a preliminary communication, see: Ward, D. E.; Vazquez, A.; Pedras, M. S. C. *J. Org. Chem*. **1996**, *61*, 8008−8009.

linkages in **1** as potential sites for cyclization because lactamization is favored over lactonization and cyclization between residues of opposite absolute configuration is particularly favorable.[18] The two five-residue linear precursors associated with these disconnections each presents the opportunity for convergent synthesis via a [2 + 3] fragment coupling approach because the Aba-Hpp linkage can be formed without the usual risk of *C*-terminal epimerization. This strategy also allows for flexibility in the choice of an Aba precursor (e.g., D- or L-threonine or *allo*-threonine)[19] should it be necessary to generate this residue later in the synthesis. Despite the greater steric hindrance, we chose D-Leu-Val site for cyclization because (i) comparing the fragments required for preparation of the two possible five-residue linear precursors, i.e., Val-Aba + Hpp-Hmp-D-Leu versus D-Leu-Val-Aba + Hpp-Hmp, the former could be prepared by more direct syntheses with less need for protecting groups; (ii) if the use of an Aba surrogate was required, it would be easier to prepare dipeptides with alternate *C*-terminal residues (i.e., Val-Xxx) compared to tripeptides (i.e., D-Leu-Val-Xxx). Thus our synthetic plan required the preparation of an *N*-protected Val-(*E*)-Aba dipeptide and a *C*-protected Hpp-Hmp-D-Leu tridepsipeptide followed by fragment coupling, deprotection, and cyclization; because orthogonal protecting groups could be unnecessary, our initial synthetic targets were (*E*)-**5** and **6**.

The three component residues of the tridepsipeptide **6** are readily available. Silylation[20a] of Hpp-OH[21] gave **11** (Scheme 2). Condensation of Hmp-OH (**8**)[22] with the *p*-toluenesulfonic acid salt of D-Leu-OBn (**9**)[23] mediated by BOP[24] gave the didepsipeptide **10** (90%)[25] which, in turn, was acylated with TBSO-Hpp-Cl (**12**; generated[20] in situ from **11**) in the presence of DMAP to provide **7** in 95% yield. Removal of the silyl ether protecting group in **7** (HF, CH_3CN; 93%) gave the desired the tridepsipeptide fragment Hpp-Hmp-D-Leu-OBn (**6**).

Scheme 2



Condensation of 2-oxobutanoic acid (**13**) with Cbz-Val-NH_2 (**14**)[26] gave the dipeptide fragment Cbz-Val-Aba (**5**) as a separable 4:1 mixture of (*Z*)-**5** and (*E*)-**5**, respectively, in good yield after optimization of the general procedure of Rzeszotarska et al.[27] (Scheme 2). The Aba configurations were assigned[28] on the basis of positive NOE correlations observed between the vinyl methyl group and the enamine N−H in (*Z*)-**5** and between the vinyl proton and the enamine N−H in (*E*)-**5** and are in accord with the thermodynamic preference for the (*Z*)-isomer established in similar examples.[7,27,29] Although this procedure has been reported to give racemic dipeptides in some examples,[27b] **5** was obtained with at least 80% enantiomeric purity under our conditions.[30]

Initial attempts to couple (*Z*)-**5** or (*E*)-**5** with **6** using DCC/DMAP gave highly variable yields (35−75%) of (*Z*)-**15** as the only isolated product. To further investigate

(17) (a) Cavelier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, J.; Pèpe, G. *J. Mol. Struct.* (*THEOCHEM*) **1993**, *286*, 125−130. (b) Cavelier-Frontin, F.; Pèpe, G.; Verducci, J.; Siri, D.; Jacquier, J. *J. Am. Chem. Soc.* **1992**, *114*, 4, 8885−8890. (c) Ueda, K.; Waki, M.; Izumiya, N. *Int. J. Pept. Protein Res.* **1987**, *30*, 33−39, and cited references.

(18) (a) Brady, S. F.; Varga, S. L.; Friedinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101−3105. (b) Kessler, H.; Kutscher, B. *Liebigs Ann. Chem.* **1986**, 869−892. (c) Kessler, H.; Kutscher, B. *Liebigs Ann. Chem.* **1986**, 914−931. (d) Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831−8838.

(19) Changing the configuration of one residue in the linear precursor can have a dramatic effect on cyclization.[11c,12,17]

(20) (a) Wissner, A.; Grudzinskas, C. V. *J. Org. Chem.* **1978**, *43*, 3972−3974. (b) Ward, D. E.; Rhee, C. K. *Tetrahedron Lett.* **1991**, *32*, 7165−7166.

(21) Urban, F. J.; Moore, B. S. *J. Heterocycl. Chem.* **1992**, *29*, 431−438.

(22) Degerbeck, F.; Fransson, B.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1* **1993**, 11−14.

(23) Miller, H. K.; Waelsch, H. *J. Am. Chem. Soc.* **1952**, *74*, 1092−1093.

(24) Abbreviations for reagents: AIBN, 2,2′-azobis(2-methylpropanenitrile); $(Boc)_2O$, bis(1,1-dimethylethyl) dicarbonate; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BOP-Cl, *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; CSA, 10-camphorsulfonic acid; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DEAD, diethyl azodicarboxylate; DMAP, 4-(dimethylamino)pyridine; DPPA, diphenylphosphoryl azide; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; FDPP, pentafluorophenyl diphenylphosphinate; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid.

(25) Similar results[16] were obtained using DCC/HOBt in place of BOP and/or with the hydrochloride salt of D-Leu-OBn.[23]

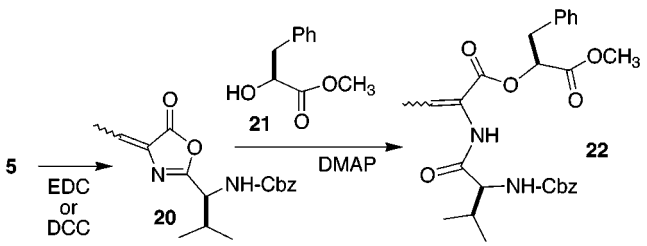
(26) Prepared from Cbz-Val according to Wang's procedure: Chen, S.-T.; Wu, S.-H.; Wang, K.-T. *Synthesis* **1989**, 37−38.

(27) (a) Makowski, M.; Rzeszotarska, B.; Kubica, Z.; Wieczorek, P. *Liebigs Ann. Chem.* **1984**, 920−928. (b) Smelka, L.; Rzeszotarska, B.; Pietrznski, G.; Kubica, Z. *Liebigs Ann. Chem.* **1988**, 485−486.

(28) Shimohigashi, Y.; Nitz, T. J.; Stammer, C. H. *Tetrahedron Lett.* **1982**, *23*, 3235−3236.

(29) (a) Srinivasan, A.; Stephensen, R. W.; Olsen, R. K. *J. Org. Chem.* **1977**, *42*, 2256−2260. (b) Nitz, T. J.; Holt, E. M.; Rubin, B.; Stammer, C. H. *J. Org. Chem.* **1981**, *46*, 2667−2673. (c) Goodall, K.; Parsons, A. F. *Tetrahedron Lett.* **1995**, *36*, 3259−3260.

Scheme 3



this process, the condensation of **5** with **21** was examined in detail (Scheme 3). Reaction of **5** with EDC (or DCC) at 0 °C rapidly gave the isolable 4-ethylidene-5(4*H*)-oxazolone **20** as a 2:1 mixture of (*Z*)-:(*E*)-isomers (32% yield) from (*E*)-**5** or as the (*Z*)-isomer (90% yield) from (*Z*)-**5**.[31] In $CDCl_3$ solution, reaction of (*Z*)-**20** with **21** occurred very slowly if at all (by ^{1}H NMR) in the presence or absence of DMAP; however, the coupling product **22** could be detected immediately after concentration of the reaction mixture. The adduct **22** was obtained as a separable 4:1 mixture of (*Z*)-:(*E*)-isomers in 60% yield from (*Z*)-**20** by allowing the concentrated reaction mixture to stand at room temperature for 30 min prior to fractionation.[32] Similarly, reaction of (*Z*)-**20** or a 2:1 mixture of (*Z*)-**20**:(*E*)-**20** with **6** under these conditions gave, in each case, an approximately 10:1 mixture of (*Z*)-**15** and (*E*)-**15**, respectively (45−60% yield).[31] These results clearly indicated that isomerization of the Aba residue in **5** occurs both during formation of the oxazolone and during acylation[33] and suggested that incorporation of the desired (*E*)-configuration would require a stereoselective isomerization of a (*Z*)-isomer at a later stage in the synthesis. The yield of the coupling reaction could be improved by avoiding the isolation of the oxazolone **20** and, under optimized conditions, the DCC/DMAP-mediated condensation of **6** with the 4:1 mixture of (*Z*)-**5**:(*E*)-**5** gave **15** as a 10−12:1 mixture of (*Z*)- and (*E*)-isomers, respectively, in 91% yield.

Chemoselective removal of the benzyl ester and carbamate protecting groups in the presence of the olefin in **15** required reduction under catalytic transfer hydrogenation conditions[34] to give the putative amino acid **16**. Cyclization of **16** using various coupling reagents under standard conditions (ca. 1 mM) gave isophomalide (**2**) in modest to good overall yields from **15**: BOP−Cl[35] (20%), DPPA[36] (31%), FDDP[37] (48%), BOP[38] (57%). Improved yields and better reproducibility were obtained using the pentafluorophenyl-activated ester method for cyclization.[39] Thus, hydrogenolysis of **15** in the presence[40] of di-*tert*-butyl dicarbonate gave **17** (90%) which was converted into the corresponding pentafluorophenyl ester **18** (85%). The Boc group in **18** was removed by treatment with TFA, and the resulting trifluoroacetate salt was added to a two-phase mixture of $CHCl_3$ and aqueous $NaHCO_3$ to give **2** (82%; 62% from **15**).[39b,c] Isophomalide (**2**) was clearly differentiated from phomalide (**1**) by NMR,[41] by HPLC,[42] and other properties (vide infra). For example, conversion of **1** to **4** occurs in aqueous methanol solution within 24 h at ambient temperature; under the same conditions, **2** is converted into (*Z*)-**4** with a half-life of >30 days.

Initial attempts to isomerize (*Z*)-**15** were unsuccessful, and this approach was abandoned after discovering that cyclization of (*E*)-**15**[43] via the pentafluorophenyl ester as described above gave a 1.2:1 mixture of **2** and **1**, respectively in 64% yield. Numerous attempts to isomerize **2** into **1** under acidic (TFA, $CDCl_3$, rt; CSA, THF, 66 °C), basic (Et_3N, $CDCl_3$, rt; DBU, benzene, rt; NaH, THF, rt; BuLi, THF, −78 °C), free radical ($HSi(SiMe_3)_3$, AIBN, PhH, 80 °C),[44] or photochemical conditions (PhSSPh, $h\nu$)[45] failed. Conjugate addition of $PhCH_2SeH$ (or PhSeH) to (*Z*)-isomers of simple α,β-unsaturated amino acid derivatives followed by oxidation has been reported to give the corresponding (*E*)-isomers with fair to good stereoselectivity (2−10:1).[46] Reaction of **2** with excess PhSeH (3 equiv) and NaOMe (0.05 equiv) in refluxing THF according to the procedure of Mazur and Pilipauskas[46] gave a 2.5:1 mixture of **19a** and **19c** in 38% yield along with recovered **2** (28%); alternatively, the use of BuLi (0.05 equiv) as base under the same conditions gave the readily separable selenides **19a** (52%), **19b** (12%), and **19c** (6%). Oxidation of **19a** with H_2O_2 gave phomalide (**1**; 94%) uncontaminated with isophomalide (**2**).[47,48] Similar reac-

(30) For related examples, the reported[27b] reaction conditions involve refuxing in benzene for 4−10 h with p-TsOH (0.2 equiv). In our case, we examined various conditions and found that refluxing in toluene for 20−30 min gave improved yields and a much cleaner reaction. Hydrolysis of **5** (obtained under optimized conditions) by heating overnight in HCl/HOAc/H_2O gave **14** of 80% ee as determined by optical rotation. That 80% ee is the minimum enantiopurity of **5** is further supported by obtention of (*Z*)-**15** and (*E*)-**15** in >90% combined yield; other diastereomers were not detected.

(31) The stereochemistry of **20** was readily assigned by ^{1}H NMR in analogy with related compounds: (a) Arenal, I.; Bernabe, M.; Fernadez-Alvarez, E. *An. Quim., Ser. C* **1981**, *77*, 56−62. (b) Cativiela, C.; Diaz De Villegas, M. D.; Mayoral, J. A.; Melendez, E. *Synthesis* **1983**, 899−902.

(32) The configurations of the Aba residues in (*Z*)-**22** and (*E*)-**22** were assigned by ^{1}H NMR on the basis of the well-documented trend that the chemical shifts for the vinyl proton, the vinyl methyl group, and the enamine N−H are at lower fields in the (*E*)-isomers than in the (*Z*)-isomers. For example, see: (a) Srinivasan, A.; Richards, K. D.; Olsen, R. K. *Tetrahedron Lett.* **1976**, 891−894. (b) Shin, C.; Hayakawa, M.; Suzuki, T.; Ohtsuka, A. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 550−554.

(33) Isomerization during acylation with *C*-terminal α,β-unsaturated amino acids is precedented: Makowski, M.; Rzezotarska, B.; Kubica, Z.; Pietrzynski, G.; Hetper, J. *Liebigs Ann. Chem.* **1986**, 980−991.

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(41) Small differences in the chemical shifts of the signals for the Aba residues were apparent; otherwise, the ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of **2** ($\Delta\delta_H < 0.1$; $\Delta\delta_C < 0.5$).

(42) Retention times for **1** and **2** were 30.4 and 29.8 min, respectively, with a hypersil ODS column (5-μm particle size; 4.6 × 200 mm) using MeCN/water gradient elution (1 mL/min; 25−75% MeCN over 35 min).

(43) Obtained in 8% yield by careful fractionation (SiO_2; benzene/ether/acetone, 90:5:5) of a 10:1 mixture of (*Z*)-**15** and (*E*)-**15**.

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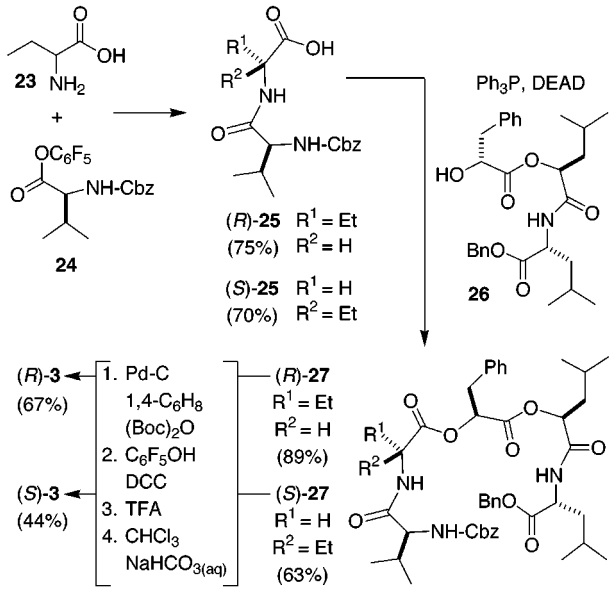
Scheme 4



tion of **19c** also gave **1** (85%) exclusively but only **2** (90%) was obtained from oxidation of **19b**. Synthetic phomalide was identical in all respects (^{1}H and ^{13}C NMR, $[\alpha]_D$, TLC and HPLC retention times) with an authentic sample.

The dihydrophomalide analogues (*R*)-**3** and (*S*)-**3** were prepared from 2-aminobutanoic acid (**23**) as shown in Scheme 4. Reactions of **24** with (*R*)-**23** and (*S*)-**23** gave the dipeptide acids (*R*)-**25** (75%) and (*S*)-**25** (70%) which were separately esterified (with inversion of configuration) with the tridepsipeptide alcohol **26**[49] via a Mitsunobu reaction[50] to give (*R*)-**27** (89%) and (*S*)-**27** (63%), respectively. The latter fragment coupling procedure ensured the configurational fidelity of the 2-aminobutanoic acid (Abu) residue. Cyclizations of (*R*)-**27** and (*S*)-**27** using the pentafluorophenyl-activated ester method gave (*R*)-**3** (67%) and (*S*)-**3** (44%), respectively. The considerably higher yields obtained in (*R*)-series reflect the greater facility of preparation and cyclization of (depsi)peptides containing adjacent residues with opposite absolute configurations.[11c,13,17] The dihydrophomalide diastereomers **3** are readily distinguished by NMR and have very different chromatographic mobilities.[51]

Hydrogenation of either **1** or **2** over Pd−C gave (*S*)-**3** diastereoselectively (<5% (*R*)-**3**). Alternatively, reduction (Ph_3SnH, AIBN)[52] of the selenides **19a** or **19b** gave (*S*)-**3** (85%); similar reduction of **19c** gave (*R*)-**3** (80%). Because elimination of selenides via the corresponding selenoxides is known to proceed with syn stereoselectivity,[53] these latter results established the absolute configurations for the selenides **19**. Thus, a (2*R*,3*R*)-configuration can be assigned for the 2-amino-3-(phenylselenyl)butanoic acid residue in **19a** because reduction gives (*S*)-**3** and oxidation gives **1**; by similar reasoning, the same residues in **19b** and **19c** are assigned the (2*R*,3*S*)- and (2*S*,3*S*)-configurations, respectively. Interestingly, addition of PhSeH to **1** gave a mixture of the selenides **19** (**19a**:**19b**:**19c** = 8:2.5:1 by HPLC) in a ratio similar to that obtained from **2** (**19a**:**19b**:**19c** = 7.5:1.5:1 by HPLC). These results indicate that preferential formation of the *like* diastereomer on addition of PhSeH to the Aba residues in **1** and **2** under these conditions is thermodynamically controlled.[54] By contrast, although the *like* diastereomer predominated in all cases, addition of selenols to (*E*) versus (*Z*)-isomers of simple α,β-unsaturated amino acid derivatives reportedly gives very different ratios of adduct diastereomers suggesting kinetically controlled stereoselectivity.[46] Nonetheless, the diastereoselective transformation of a (*Z*)-α,β-unsaturated amino acid residue into the corresponding (*E*)-isomer via the *like* β-selenide derivative appears to have useful scope. Considering the mildness of this method and the relatively straightforward access to peptides containing (*Z*)-α,β-unsaturated amino acid residues,[7,13] isomerization of those peptides is an attractive strategy for the synthesis of the elusive (*E*)-isomers.

We have evaluated the phytotoxicity of phomalide (**1**), its (*Z*)-isomer (isophomalide, **2**), and the two dihydro derivatives (*R*)-**3** and (*S*)-**3** to plants susceptible (canola) and resistant [brown mustard (*Brassica juncea*) and white mustard (*Sinapis alba*)] to blackleg, as previously reported for other toxins.[55] The naturally occurring phomalide (**1**) caused necrotic, chlorotic, and reddish lesions on canola leaves (10^{-5} M), whereas no damage was observed on brown or white mustard leaves, even at significantly higher concentrations (10^{-4} M). Thus, the selective phytotoxicity of phomalide (**1**) appears to mimic the pathogenicity range of the blackleg fungus; i.e., phomalide causes lesions on plants that are susceptible to blackleg infection (canola), but not on resistant plants (brown and white mustards). Most importantly, the (*Z*)-isomer **2** did not cause lesions on any of the plant leaves tested, even at 5×10^{-4} M. Interestingly, however, both dihydrophomalides (*R*)-**3** and (*S*)-**3** (5×10^{-5} M) caused chlorotic lesions on brown mustard but not on canola or white mustard. These results indicate that both the presence and configuration of the double bond in phomalide (**1**) are important for selective phytotoxicity; however, it remains to be determined if and how resistant plants enzymatically convert **1** to less toxic products. Preliminary results indicate that phomalide (**1**) is metabolized by brown mustard leaf tissue to a significantly more polar compound.

In summary, we have achieved an efficient synthesis of the host-selective phytotoxin phomalide (**1**) and, thereby, the first synthesis of an (*E*)-Aba-containing natural

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(48) Phomalide and isophomalide are separable (PTLC; 30% ethyl acetate in hexane, multiple development), albeit with considerable difficulty (phomalide is slightly less polar) and with low efficiency (i.e., mainly mixed fractions are obtained).

(49) Obtained in 90% yield from **10** and (2*R*)-2-hydroxy-3-phenylpropanoic acid in analogy to the preparation of **6**.

(50) (a) Lee, B. H. *Tetrahedron Lett.* **1997**, *38*, 757−760. (b) Ohyama, M.; Iinuma, K.; Suzuki, A. *Biosci. Biotech. Biochem.* **1994**, *58*, 1193−1194. (c) Schmidt, U.; Langner, J. *J. Chem. Soc., Chem. Commun.* **1994**, 2381−2382.

(51) TLC, SiO_2 (50% ethyl acetate in hexane): (*R*)-**3**, $R_f = 0.67$; (*S*)-**3**, $R_f = 0.26$.

(52) Clive, D. L. J.; Chittattu, G. J.; Farina, V.; Kiel, W. A.; Menchen, S. M.; Russell, C. G.; Singh, A.; Wong, C. K.; Curtis, N. J. *J. Am. Chem. Soc.* **1980**, *102*, 4438−4447.

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(54) Addition of PhSeH to Aba residues in acyclic peptides also gives the *like* diastereomer selectively. For example reactions of Val-(*Z*)-Aba-Hpp-Hmp derivatives with PhSeH/BuLi followed by oxidation gave the corresponding (*E*)-isomers with ca. 5:1 diastereoselectivity (Ward, D. E.; Vázquez, A. Unpublished results).

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product. The key step in the synthesis involved a diastereoselective isomerization of the (*Z*)-isomer isophomalide (**2**), itself prepared in 45% overall yield in 6 steps from the readily available **8** and **9** via a [2 + 3] fragment coupling approach followed by cyclization. The strategy is suitable for the preparation of analogues as demonstrated by the synthesis of (*R*)-**3** and (*S*)-**3** and should be amenable to the preparation of radiolabeled congeners. Importantly, this (*Z*) → (*E*) isomerization approach should be applicable to other (depsi)peptide targets thereby allowing an investigation of the effect of the double-bond configuration on various properties. As illustrated with **1** and **2**, the double-bond configuration can have a remarkable influence on chemical and biological properties, particularly phytotoxicity.

Experimental Section

General Methods. All solvents were distilled prior to use. Unless otherwise noted, reactions were carried out under an atmosphere of argon and reaction temperatures refer to the bath. Concentration refers to removal of volatiles at water aspirator pressure on a rotary evaporator. Preparative thin-layer chromatography (PTLC) was carried out on glass plates (20 × 20 cm) precoated (0.25 mm) with silica gel 60 F_{254}. Flash column chromatography (FCC), dry flash column chromatography (DFC), and medium-pressure chromatography (mpc) were performed according to the procedures of Still et al.,[56] Harwood,[57] and Taber,[58] respectively. All mixed solvent eluents are reported as volume-to-volume (v/v) solutions.

Spectral Data and Analysis. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were obtained on a double focusing high-resolution spectrometer; only partial data are reported. Electron impact (EI) ionization was accomplished at 70 eV, chemical ionization (CI) at 50 eV with ammonia as the reagent gas, and fast-atom bombardment (FAB) in positive ion mode from a glycerol and MeOH matrix. Infrared spectra were recorded on a Fourier transform interferometer using a diffuse reflectance cell (DRIFT); only diagnostic peaks are reported. Unless otherwise noted, NMR spectra were measured in $CDCl_3$ solution at 300 MHz for ^{1}H and 75 MHz for ^{13}C. The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), ap (apparent). The list of coupling constants (*J*; reported to the nearest 0.5 Hz) corresponds to the order of the multiplicity assignment. Optical rotations ($[\alpha]_D$) were determined at ambient temperature using a 1 mL, 10 dm cell; the units are 10^{-1} deg cm^2 g^{-1}, and the concentrations (*c*) are reported in g/100 mL.

Materials. (*R*)- and (*S*)-Hpp,[21] (*S*)-Hmp,[22] D-Leu-OBn·*p*-TsOH,[23] Boc-Val, and Cbz-Val were prepared from the corresponding amino acids according to literature procedures. All other reagents were commercially available and, unless otherwise noted, were used as received.

Phomalide [cyclo(Val-(*E*)-Aba-Hpp-Hmp-(*R*)-Leu)] (1). From **19a**: 30% H_2O_2 (0.100 mL, 1.16 mmol) was added via syringe to a solution of selenide **19a** (98 mg, 0.14 mmol) in acetone (2 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then diluted with water and extracted with ethyl acetate (×2). The combined organic layers was washed with brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (30% ethyl acetate in hexane) to give phomalide (**1**) (72 mg, 94%) as a white solid which was identical in all respects with an authentic sample. From **19c**: Oxidation of **19c** (6 mg, 0.008 mmol) as above with 30% H_2O_2 (0.025 mL) gave **1** (4 mg, 85%): mp 155−156 °C (CH_2Cl_2/hexane), 165−168 °C (MeCN, H_2O); $[\alpha]_D$ −48 (*c* 0.19, $CHCl_3$); IR ν_{max} 3282, 3064, 1747, 1693, 1665, 1649 cm^{-1}; ^{1}H NMR (500 MHz) δ 8.00 (1H, br s), 7.30−7.23 (5H, m), 6.80 (1H, q, *J* = 7.5 Hz), 6.40 (1H, d, *J* = 8 Hz), 6.39 (1H, d, *J* = 9 Hz), 5.39 (1H, dd, *J* = 5, 8 Hz), 5.33 (1H, dd, *J* = 4, 9 Hz), 4.38 (1H, ddd, *J* = 7, 8, 8 Hz), 4.24 (1H, dd, *J* = 9, 9 Hz), 3.30 (1H, dd, *J* = 4, 14.5 Hz), 3.16 (1H, dd, *J* = 9, 14.5 Hz), 2.26−2.19 (1H, m), 1.83 (3H, d, *J* = 7.5 Hz), 1.80−1.75 (1H, m), 1.74−1.69 (1H, m), 1.61−1.54 (2H, m), 1.53−1.48 (1H, m), 1.36−1.30 (1H, m), 0.99 (3H, d, *J* = 6.5 Hz), 0.94 (3H, d, *J* = 7 Hz), 0.92 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 0.83 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz) δ 172.3, 170.0, 169.0, 168.2, 166.0, 135.0, 131.8, 129.3 (×2), 128.6 (×2), 127.4, 124.5, 76.1, 72.6, 59.0, 52.1, 39.2, 39.2, 37.0, 27.2, 24.9, 24.1, 22.9, 22.4, 22.3, 21.8, 19.9, 18.0, 14.4; LRMS (EI) *m*/*z* (rel intensity) 557 ([M]$^+$, 72), 72 (100); HRMS *m*/*z* calcd for $C_{30}H_{43}N_3O_7$ 557.3101, found 557.3092 (EI). Anal. Calcd for $C_{30}H_{43}N_3O_7$·0.2H_2O: C, 64.20; H, 7.79; N, 7.49. Found: C, 64.04; H, 7.61; N, 7.42.

Isophomalide [cyclo(Val-(*Z*)-Aba-Hpp-Hmp-(*R*)-Leu)] (2). From **19b**: Oxidation of **19b** (3 mg, 0.004 mmol) with 30% H_2O_2 (0.025 mL, 1.16 mmol) in acetone (0.5 mL) at 0 °C as described above gave **2** (2 mg, 90%). From **18**: To a stirred solution of the pentafluorophenyl ester **18** (308 mg, 0.365 mmol) in CH_2Cl_2 (6 mL) at 0 °C was added TFA (2 mL). After 1 h, the mixture was concentrated, and the residue was evacuated at high vacuum (0.5 Torr) for 1 h. The residue was dissolved in CH_2Cl_2 (7 mL), and the solution was added over 5 min to a vigorously stirred mixture of $CHCl_3$ (7 mL) and saturated $NaHCO_3$(aq) (7 mL). Stirring was continued for 2.5 h, and then the aqueous layer was diluted with brine and extracted with CH_2Cl_2 (×2). The combined organic layers was dried over Na_2SO_4, concentrated, and fractionated by FCC (30% ethyl acetate in hexane) to give **2** as a white solid (167 mg, 82%; 62% overall yield from **15**): mp 168−169 °C (CH_2Cl_2/hexane); $[\alpha]_D$ −44 (*c* 0.14, $CHCl_3$); IR ν_{max} 3334, 3282, 1744, 1727, 1683, 1651 cm^{-1}; ^{1}H NMR (500 MHz) δ 7.73 (1H, s), 7.32−7.24 (5H, m), 6.67 (1H, q, *J* = 7 Hz), 6.55 (1H, d, *J* = 8.5 Hz), 6.21 (1H, d, *J* = 10 Hz), 5.43 (1H, dd, *J* = 4, 9.5 Hz), 5.14 (1H, dd, *J* = 4, 8.5 Hz), 4.41 (1H, ddd, *J* = 7.5, 8, 8.5 Hz), 4.23 (1H, dd, *J* = 10, 10 Hz), 3.25 (1H, dd, *J* = 14.5, 4 Hz), 3.16 (1H, dd, *J* = 14.5, 8.5 Hz), 2.19−2.11 (1H, m), 1.82−1.77 (1H, m), 1.80 (3H, d, *J* = 7 Hz), 1.71−1.65 (2H, m), 1.59−1.45 (3H, m), 1.15−1.08 (1H, m), 0.98 (3H, d, *J* = 6.5 Hz), 0.96 (3H, d, *J* = 7 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 0.90 (3H, d, *J* = 6.5 Hz), 0.78 (3H, d, *J* = 6.5 Hz), 0.77 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz) δ 172.0, 169.5, 169.1, 168.1, 166.3, 135.2 (×2), 129.5 (×2), 128.6 (×2), 127.4, 125.5, 75.5, 72.4, 59.3, 51.8, 39.3, 39.3, 37.0, 27.0, 24.9, 23.8, 23.1, 22.5, 22.4, 21.3, 19.7, 18.3, 15.3; LRMS (FAB) *m*/*z* (rel intensity) 558 ([M + 1]$^+$, 34), 119 (100); HRMS *m*/*z* calcd for $C_{30}H_{43}N_3O_7$+H 558.3179 (M + H), found 558.3186 (FAB). Anal. Calcd for $C_{30}H_{43}N_3O_7$: C, 64.61; H, 7.77; N, 7.53. Found: C, 64.38; H, 7.86; N, 7.33.

(*R*)-Dihydrophomalide [cyclo(Val-(*R*)-Abu-Hpp-Hmp-(*R*)-Leu)] [(*R*)-3]. From **19c**: A stirred solution of Ph_3SnH (8 mg, 0.024 mmol), **19c** (4 mg, 0.006 mmol), and AIBN (a small crystal) in toluene (0.50 mL) was heated under reflux under argon. After 16 h, the cooled (rt) reaction mixture was concentrated and fractionated by MPC (SiO_2, 1.5 g; 50% ethyl acetate in hexane) to give (*R*)-**3** (2.7 mg, 80%). From (*R*)-**27**: A mixture of (*R*)-**27** (93 mg, 0.12 mmol) and 10% Pd/C (10 mg) in 95% EtOH (1.5 mL) was stirred under hydrogen (1 atm) at ambient temperature for 1 h. The mixture was filtered through Celite, and the combined filtrate and washings was concentrated to give a foam (82 mg). DCC (25 mg, 0.12 mmol) was added to a stirred solution of the above foam and C_6F_5OH (22 mg, 0.12 mmol) in ethyl acetate (0.6 mL) at 0 °C. After 1 h of stirring, the mixture was filtered, and the combined filtrate and washings was concentrated and fractionated by FCC (SiO_2; 25% ethyl acetate in hexane) to provide the pentafluorophenyl ester as a foam (85 mg, 83%). TFA (1.0 mL) was added to a stirred solution of the active ester (57 mg, 0.066 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. After 90 min, the mixture was concentrated and reconcentrated twice from toluene. The residue was dissolved in CH_2Cl_2 (2.0 mL), and the solution was added dropwise via syringe over 5 min to a vigorously stirred mixture

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(58) Taber, D. F. *J. Org. Chem.* **1982**, *47*, 1351−1352.

of $CHCl_3$ (2.0 mL) and saturated $NaHCO_3$(aq) (2.0 mL). After 12.5 h of stirring at room temperature, the two phases were separated. The aqueous layer was diluted with brine and extracted with ethyl acetate (×3). The combined organic layers was washed with brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (SiO_2; 30% ethyl acetate in hexane) to provide (*R*)-**3** as a white solid (31 mg, 84%, 70% overall from (*R*)-**27**): $[\alpha]_D$ −19 (*c* 0.8, $CHCl_3$); IR ν_{max} 3376, 3258, 3068, 1737, 1680, 1642 cm^{-1}; ^1H NMR (500 MHz) δ 7.2−7.3 (5H, m), 6.50 (1H, d, *J* = 9.5 Hz), 6.27 (1H, d, *J* = 9 Hz), 5.99 (1H, d, *J* = 10 Hz), 5.42 (1H, dd, *J* = 4.5, 9 Hz), 5.15 (1H, dd, *J* = 3.5, 9 Hz), 4.60 (1H, ddd, *J* = 7, 7.5, 9.5 Hz), 4.49 (1H, ddd, *J* = 7.5, 7.5, 9 Hz), 4.04 (1H, ap t, *J* = 10, 10 Hz), 3.25 (1H, dd, *J* = 3.5, 14.5 Hz), 3.14 (1H, dd, *J* = 9, 14.5 Hz), 2.13−2.06 (1H, m), 1.79−1.45 (8H, m), 0.95 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 6.5 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.82 (3H, d, *J* = 6.5 Hz), 0.75 (3H, t, *J* = 7.5 Hz); ^{13}C NMR (125 MHz) δ 175.2, 172.75, 171.6, 169.5, 168.1, 135.4, 129.7 (×2), 128.8 (×2), 127.6, 75.4, 72.8, 59.6, 53.3, 51.2, 39.5, 39.0, 37.1, 27.4, 26.0, 25.0, 24.3, 23.3, 22.9, 22.5, 21.8, 19.9, 18.7, 10.0; LRMS (EI) *m*/*z* (rel intensity) 559 ($[M]^+$, 24), 72 (100); HRMS *m*/*z* calcd for $C_{30}H_{45}N_3O_7$ 559.3258, found 559.3260 (EI). Anal. Calcd for $C_{30}H_{45}N_3O_7$: C, 64.38; H, 8.10; N, 7.51. Found: C, 64.48; H, 7.88; N, 7.48.

(*S*)-Dihydrophomalide [cyclo(Val-(*S*)-Abu-Hpp-Hmp-(*R*)-Leu)] [(*S*)-3]. From **1**: A mixture of phomalide (15 mg, 0.026 mmol) and 10% Pd/C (5 mg) in ethyl acetate (1.0 mL) was stirred under H_2 (1 atm) at room temperature for 3 h. The mixture was filtered, and the combined filtrate and washings was concentrated to give (*S*)-**3** (15 mg, 100%). From **2**: Isophomalide (61 mg, 0.11 mmol) was hydrogenated over 10%Pd/C (12 mg) as above to give (*S*)-**3** (51 mg, 84%). From **19a**: A stirred solution of PH_3SnH (20 mg, 0.056 mmol), **19a** (10 mg, 0.014 mmol), and AIBN (a small crystal) in toluene (0.50 mL) was heated under reflux under argon. After 23 h, the cooled (rt) reaction mixture was concentrated and fractionated by MPC (SiO_2, 2 g; 50% ethyl acetate in hexane) to give (*S*)-**3** (7 mg, 88%). From **19b**: Selenide **19b** (4 mg, 0.006 mmol) was reduced with Ph_3SnH (8 mg, 0.024 mmol) as described above to yield (*S*)-**3** (2.8 mg, 85%). From (S)-**27**: As described above for (*R*)-**27** → (*R*)-**3**, (*S*)-**27** (92 mg, 0.12 mmol) was converted into the corresponding pentafluorophenyl ester (94 mg, 92%). Cyclization of the active ester (57 mg, 0.066 mmol) gave (*S*)-**3** as a white solid (18 mg, 49%) after fractionation by FCC (SiO_2; 50% ethyl acetate in hexane): $[\alpha]_D$ −47 (*c* 1.0, $CHCl_3$); IR ν_{max} 3270, 3264, 1747, 1672, 1650, cm^{-1}; ^1H NMR (500 MHz) δ 7.15−7.35 (5H, m), 6.88 (1H, d, *J* = 9 Hz), 6.32 (1H, d, *J* = 9.5 Hz), 5.23 (1H, dd, *J* = 7, 7 Hz), 5.09 (1H, dd, *J* = 5, 8 Hz), 4.53 (1H, ddd, *J* = 6, 8.5, 9 Hz), 4.39 (1H, dd, *J* = 4.5, 9.5 Hz), 4.21 (1H, ddd, *J* = 7.5, 7.5, 7.5 Hz), 3.18 (1H, dd, *J* = 4.5, 14 Hz), 3.13 (1H, dd, *J* = 8.5, 14 Hz), 2.45−2.35 (1H, m), 1.98−1.77 (3H, m), 1.77−1.68 (1H, m), 1.65−1.48 (3H, m), 1.48−138 (1H, m), 0.97−0.87 (21H, m); ^{13}C NMR (125 MHz) δ 171.6, 171.4, 171.2, 171.1, 168.7, 135.3, 129.6 (×2), 128.8 (×2), 127.5, 75.4, 72.9, 59.6, 53.9, 53.3, 40.5, 38.6, 36.9, 29.3, 25.0, 24.5, 24.2, 22.9, 22.6 (×2), 22.5, 19.8, 17.3, 10.6; LRMS (EI) *m*/*z* (rel intensity) 559 ($[M]^+$, 18), 72 (100); HRMS *m*/*z* calcd for $C_{30}H_{45}N_3O_7$ 559.3258, found 559.3254 (EI). Anal. Calcd for $C_{30}H_{45}N_3O_7$: C, 64.34; H, 8.10; N, 7.51. Found: C, 64.18; H, 7.85; N, 7.42.

Hmp-(R)-Leu-Val-(E)-Aba-Hpp-OCH₃ (4). A solution of **1** (3 mg, 0.005 mmol) in MeOH (2.0 mL) and H_2O (1.0 mL) was stirred at room temperature for 29 h. The reaction mixture was concentrated and extracted with CH_2Cl_2 (×2), and the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was fractionated by PTLC (50% ethyl acetate in hexane) to provide **4** (3 mg, >90%): $[\alpha]_D$ −10 (*c* 0.4, $CHCl_3$); IR ν_{max} 3299, 1737, 1656, 1648 cm^{-1}; ^1H NMR (500 MHz) δ 8.0 (1H, s), 7.32−7.14 (6H, m), 7.10 (1H, d, *J* = 8.5 Hz), 6.93 (1H, q, *J* = 7.5 Hz), 5.37 (1H, dd, *J* = 5.5, 7 Hz), 4.49 (1H, ddd, *J* = 6, 8, 8 Hz), 4.26 (1H, dd, *J* = 6, 8.5 Hz), 4.11 (1H, ddd, *J* = 3.5, 6, 10 Hz), 3.71 (3H, s), 3.47 (1H, d, *J* = 6 Hz), 3.24 (1H, dd, *J* = 5.5, 14.5 Hz), 3.18 (1H, dd, *J* = 7, 14.5 Hz), 2.22−2.10 (1H, m), 1.96 (3H, d, *J* = 7.5 Hz), 1.71−1.46 (6H, m), 0.95−0.86 (18H, m); ^{13}C NMR (125 MHz) δ 175.7, 172.7, 170.2, 169.9, 163.8, 135.5, 131.0, 129.4 (×2), 128.8 (×2), 127.5, 125.2, 74.3, 70.9, 59.2, 52.6, 51.7, 43.7, 40.7, 37.5, 30.6, 25.0, 24.7, 23.6, 23.0, 22.3, 21.5, 19.5, 17.9, 14.5; LRMS (FAB) *m*/*z* (rel intensity) 590 ($[M + 1]^+$, 43), 72 (100); HRMS *m*/*z* calcd for $C_{31}H_{47}N_3O_8$+H 590.3441 (M + H), found 590.3413 (FAB).

Hmp-(*R*)-Leu-Val-(*Z*)-Aba-Hpp-OCH₃ [(*Z*)-4]. A solution of **2** (2.0 mg, 0.004 mmol) in MeOH (2.0 mL) and H_2O (1.0 mL) was stirred at room temperature for 56 days. The reaction mixture was concentrated, extracted with CH_2Cl_2 (×2), and the combined organic layers was dried over Na_2SO_4 and concentrated. The residue was fractionated by PTLC (50% ethyl acetate in hexane) to give recovered **2** (1 mg, 50%) and (*Z*)-**4** (1 mg, 50%): $[\alpha]_D$ −15 (*c* 0.4, $CHCl_3$); IR ν_{max} 3285, 1730, 1656, 1647, 1644 cm^{-1}; ^1H NMR (500 MHz) δ 7.57 (1H, s), 7.33−7.17 (5H, m), 7.00 (1H, d, *J* = 7 Hz), 6.89 (1H, d, *J* = 9 Hz), 6.85 (1H, q, *J* = 7 Hz), 5.25 (1H, dd, *J* = 5.5, 7 Hz), 4.44 (1H, ddd, *J* = 7, 7.5, 8 Hz), 4.37 (1H, dd, *J* = 6, 8.5 Hz), 4.09 (1H, dd, *J* = 2.5, 9 Hz), 3.70 (3H, s), 3.25−3.10 (2H, m), 2.90 (1H, bs), 2.34−2.18 (1H, m), 1.85−1.52 (5H, m), 1.73 (3H, d, *J* = 7 Hz), 1.49−1.35 (1H, m), 0.99−0.89 (18H, m); ^{13}C NMR (500 MHz) δ 175.6, 172.5, 170.2, 169.6, 163.8, 136.4, 135.7, 129.5 (×2), 128.8 (×2), 127.4, 125.5, 74.3, 70.9, 58.9, 53.5, 51.9, 43.7, 40.3, 37.6, 30.2, 25.0, 24.7, 23.6, 23.0, 22.4, 21.5, 19.7, 17.8, 15.3; LRMS (EI) *m*/*z* (rel intensity) 589 ($[M]^+$, 6), 72 (100); HRMS *m*/*z* calcd for $C_{31}H_{47}N_3O_8$: 589.3363, found 598.3364 (EI).

Cbz-Val-(*E*)-Aba-OH [(*E*)-5] and **Cbz-Val-(*Z*)-Aba-OH [(*Z*)-5].** A mixture of Cbz-Val-NH_2 (**14**; 375 mg, 1.5 mmol), 2-oxobutanoic acid (230 mg, 2.25 mmol), and *p*-TsOH·H_2O (57 mg, 0.3 mmol) in dry toluene (2.5 mL) was heated under reflux with azeotropic water removal for 30 min during which time, a precipitate formed and the solution became thick. The cooled (rt) reaction mixture was diluted with toluene, and the precipitate was filtered off and then suspended in saturated $NaHCO_3$(aq) (30 mL). The suspension was stirred for 30 min and then extracted with ethyl acetate (×4). The combined organic layers was dried over Na_2SO_4 and concentrated to give recovered **14** (120 mg, 32%). The aqueous phase was acidified (pH 3−4) with citric acid and then extracted with ethyl acetate (×4). The combined organic layers was washed with brine, dried over Na_2SO_4, and concentrated to give **5** as a white solid [a 4:1 mixture of (*Z*):(*E*) diastereomers; 232 mg, 46% (68% based on conversion)] which was used in the next step without further purification. The 4:1 mixture of isomers (190 mg) was fractionated by FCC (SiO_2; $CHCl_3$:pyridine:HOAc, 84:4:2; dry loading) to give (*E*)-**5** as a white solid (28 mg; R_f = 0.4) and (*Z*)-**5** as a white solid (120 mg; R_f = 0.26). For (*E*)-**5**: $[\alpha]_D$ +16 (*c* 0.6, DMF); IR ν_{max} 3617, 3291, 1691, 1659 cm^{-1}; ^1H NMR (500 MHz) δ 9.25 (1H, s), 7.40−7.29 (5H, m), 6.22 (2H, m), 5.03 (2H, s), 3.87 (1H, m), 1.99 (1H, m), 1.91 (3H, d, *J* = 7.5 Hz), 0.88 (3H, d, *J* = 7.0 Hz), 0.85 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz) δ 169.9, 165.4, 156.2, 137.0, 129.0, 128.4 (×2), 127.7, 127.6 (×2), 123.0, 65.4, 60.5, 30.2, 19.2, 18.1, 13.5; LRMS (Cl) *m*/*z* (rel intensity) 335 ($[M + 1]^+$, 79), 91 (100); HRMS *m*/*z* calcd for $C_{17}H_{22}N_2O_5$+H 335.1607 (M + H), found 335.1609 (FAB). For (*Z*)-**5**: $[\alpha]_D$ +24 (*c* 0.6, DMF); ^1H NMR ($(CD_3)_2SO$) δ 9.09 (1H, s), 7.36−7.21 (6H, m), 6.52 (1H, q, *J* = 7 Hz), 5.03 (2H, ap s), 3.98 (1H, dd, *J* = 7, 8.5 Hz), 2.03−1.96 (1H, m), 1.63 (3H, d, *J* = 7 Hz), 0.93 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (500 MHz, $(CD_3)_2SO$) δ 170.1, 165.4, 156.1, 137.1, 131.8, 128.3 (×2), 127.7, 127.6 (×2), 124.7, 65.4, 60.2, 30.3, 19.2, 18.1, 13.5; LRMS (CI, NH_3) *m*/*z* (rel intensity) 335 ($[M + 1]^+$, 25), 227 (100); HRMS *m*/*z* calcd for $C_{17}H_{22}N_2O_5$+H 335.1607 (M + H), found 335.1590 (FAB). Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.37. Found C, 61.26; H, 6.43; N, 8.12.

Hpp-Hmp-(*R*)-Leu-OBn (6). A solution of **7** (1.04 g, 1.73 mmol) in 5% HF/CH_3CN (20 mL) was stirred at room temperature for 2 h. The mixture was concentrated, diluted with CH_2Cl_2, washed with water, saturated $NaHCO_{3(aq)}$, and brine, dried over Na_2SO_4, and concentrated. The resulting white solid residue was fractionated by FCC (30% ethyl acetate in hexane) to afford **6** as a white solid (783 mg, 93%): mp 83−84 °C (CH_2Cl_2/hexane); $[\alpha]_D$ +2.0 (*c* 1.0, MeOH); IR ν_{max} 3491, 3408, 1734, 1652 cm^{-1}; ^1H NMR δ 7.40−7.21 (10H, m), 6.29 (1H, d, *J* = 8

Hz), 5.26 (1H, dd, J = 4.5, 8.5 Hz), 5.21−5.11 (2H, m), 4.68−4.61 (1H, ddd, J = 5, 8, 9 Hz), 4.55−4.45 (1H, m), 3.21 (1H, dd, J = 14, 4.5 Hz), 2.99 (1H, dd, J = 14, 7 Hz), 2.63 (1H, d, J = 2.5 Hz), 1.79−1.41 (6H, m), 0.96−0.87 (12H, m); ^{13}C NMR δ 173.0, 172.3, 169.4, 136.0, 135.3, 129.5, 128.6, 128.5, 128.3, 127.1, 73.9, 71.5, 67.2, 50.6, 41.4, 40.6, 40.3, 24.8, 24.4, 23.0, 22.7, 21.9, 21.7; LRMS (FAB) *m/z* (rel intensity) 484 ([M + 1]$^+$, 100); HRMS *m/z* calcd for $C_{28}H_{37}NO_6$+H 484.2699 (M + H), found 484.2714 (FAB). Anal. Calcd for $C_{28}H_{37}NO$: C, 69.54; H, 7.71; N, 2.89. Found C, 69.39; H, 7.92; N, 2.88.

TBSO-Hpp-Hmp-(*R*)-Leu-OBn (7). Oxalyl chloride (0.61 mL, 7.0 mmol) was added dropwise into a solution of **11** (2.37 g, 6.0 mmol) and DMF (0.043 mL, 0.60 mmol) in CH_2Cl_2 (8 mL) at 0 °C under argon. After 0.5 h of stirring for at 0 °C and a further 4 h at room temperature, the reaction mixture was diluted with dry hexane (10 mL), and the resulting solid was removed by filtration. The combined filtrate and washings was concentrated, and a solution of **10** (1.68 mg, 5.0 mmol) and DMAP (0.733 g, 6.0 mmol) in CH_2Cl_2 (8 mL) was added. After 2 h of stirring at room temperature, the reaction mixture was diluted with a 1:1 mixture of hexane and ethyl acetate and then was washed sequentially with water, 10% citric acid, saturated $NaHCO_3$(aq), and brine, dried over Na_2SO_4, and concentrated. The resulting yellow oil was fractionated by FCC (10% ethyl acetate in hexane) to give **7** as a colorless oil (2.84 g, 95%): $[\alpha]_D$ −26 (*c* 1.0, $CHCl_3$); IR ν_{max} 3424, 3326, 1753, 1743, 1691, 1667 cm^{-1}; ^{1}H NMR δ 7.35−7.21 (5H, m), 6.31 (1H, d, J = 8.5 Hz), 5.28 (1H, dd, J = 4, 9 Hz), 5.21−5.11 (2H, m), 4.68−4.65 (1H, ddd, J = 5, 8.5, 8.5 Hz), 4.43 (1H, dd, J = 4, 8.5 Hz), 3.13 (1H, dd, J = 4, 13.5 Hz), 2.93 (1H, dd, J = 8.5, 13.5 Hz), 1.77−1.46 (6H, m), 0.93−0.87 (12H, m), 0.81 (9H, s), −0.08 (3H, s), −0.22 (3H, s); ^{13}C NMR δ 172.3, 171.9, 169.7, 136.9, 135.3, 129.8, 128.6, 128.4, 128.3, 128.3, 126.8, 73.5, 73.1, 67.1, 50.5, 41.6, 41.4, 40.7, 25.6, 24.8, 24.4, 23.1, 22.7, 21.9, 21.6, 18.1, −5.2, −5.7; LRMS (FAB) *m/z* (rel intensity) 598 ([M + 1]$^+$, 49), 91 (100); HRMS *m/z* calcd for $C_{34}H_{51}NSiO_6$+H 598.3564 (M + H), found 598.3571 (FAB). Anal. Calcd for $C_{34}H_{51}NSiO_6$: C, 68.30; H, 8.59; N, 2.34. Found C, 68.58; H, 8.72; N, 2.32.

Hmp-(*R*)-Leu-OBn (10). Diisopropylethylamine (1.74 mL, 10.0 mmol) was added dropwise to a stirred mixture of Hmp (0.661 g, 5.0 mmol), (*R*)-Leu-OBn·p-TsOH (**9**; 1.97 g, 5.0 mmol), and BOP (2.21 g, 5.0 mmol) in MeCN (33 mL) at room temperature under argon. After 1.5 h, the reaction mixture was diluted with brine and extracted with ethyl acetate (×3). The combined organic layers was washed sequentially with 10% citric acid and brine, dried over Na_2SO_4, concentrated, and fractionated by DFC (gradient elution, 5−35% ethyl acetate in hexane) to afford **10** as a white solid (1.51 g, 90%): mp 103−104 °C ($CHCl_3$/petroleum ether); $[\alpha]_D$ +8.4 (*c* 1.1, MeOH); IR ν_{max} 3545, 3292, 1711, 1641 cm^{-1}; ^{1}H NMR δ 7.39−7.28 (5H, m), 6.84 (1H, d, J = 8.5 Hz), 5.21−5.11 (2H, m), 4.71−4.64 (1H, m), 4.17 (1H, dd, J = 3.5, 9.5 Hz), 1.86−1.79 (1H, m), 1.71−1.48 (5H, m), 0.96−0.87 (12H, m); ^{13}C NMR δ 174.5, 172.7, 135.3, 128.6 (×2), 128.4, 128.2 (×2), 70.3, 67.1, 50.5, 43.6, 41.4, 24.9, 24.5, 23.4, 22.8, 21.8, 21.4; LRMS (CI, NH_3) *m/z* (rel intensity) 336 ([M + 1]$^+$, 21), 228 (100); HRMS *m/z* calcd for $C_{19}H_{29}NO_4$ 335.2097, found 335.2096 (FAB). Anal. Calcd for $C_{19}H_{29}NO_4$: C, 68.03; H, 8.71; N, 4.17. Found C, 68.18; H, 8.73; N, 4.01.

TBSO-Hpp-OTBS (11). TBSCl (4.16 g, 27.5 mmol) was added at once to a solution of (*S*)-phenyllactic acid (Hpp) (2.22 g, 13.3 mmol) and imidazole (2.72 g, 40.0 mmol) in DMF (8 mL), and the resulting mixture was stirred at room temperature for 21 h. The mixture was partitioned between hexane and water and the organic layer was washed with saturated $NaHCO_3$(aq) and brine, dried over Na_2SO_4, and concentrated. The resulting residue was distilled under reduced pressure to give **11** as a colorless oil (4.57 g, 87%): bp 118−120 °C (0.1 mmHg); $[\alpha]_D$ −24 (*c* 1.1, $CHCl_3$): IR ν_{max} 1740, 1617 cm^{-1}; ^{1}H NMR δ 7.30−7.20 (5H, m), 4.29 (1H, dd, J = 4, 8.5 Hz), 3.08 (1H, dd, J = 4, 13.5 Hz), 2.88 (1H, dd, J = 8.5, 13.5 Hz), 0.93 (9H, s), 0.80 (9H, s), 0.25 (3H, s), 0.19 (3H, s), −0.81 (3H, s), −0.21 (3H, s); ^{13}C NMR δ 173.2, 137.6, 129.8, 128.1, 126.5, 74.4, 41.7, 25.7, 25.6, 18.2, 17.7, −4.9, −4.9, −5.2, −5.7; LRMS (CI, NH_3) *m/z* (rel intensity) 395 ([M + 1]$^+$, 100); HRMS *m/z* calcd for $C_{21}H_{38}Si_2O_3$+H 395.2438 (M + H), found 395.2421 (CI, NH_3). Anal. Calcd for $C_{21}H_{38}Si_2O_3$: C, 63.90; H, 9.70. Found C, 63.90; H, 9.79. The (*R*)-enantiomer (*ent*-**11**) ($[\alpha]_D$ +23; c 1.1, $CHCl_3$) was obtained from (*R*)-phenyllactic acid using the same procedure.

Cbz-Val-NH$_2$ (14). EDC (3.40 g, 17.8 mmol) was added to a stirred mixture of Cbz-Val (4.26 g, 17.0 mmol) and HOBt (2.52 g, 18.6 mmol) in CH_2Cl_2 (80 mL) and DMF (20 mL) at 0 °C. After 30 min, 28% aqueous NH_3 (1.4 mL, 22 mmol) was added dropwise to the reaction mixture (precipitate formation). The resulting thick mixture was stirred for an additional 1.5 h at 0 °C and 4 h at room temperature and then was filtered. The combined filtrate and washings was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$(aq) and water, and dried over Na_2SO_4. The filter cake was triturated with boiling MeCN, and after removal of the insoluble material, the filtrate was combined with the previously obtained organic layer. Concentration gave a white solid that was recrystallized from MeCN to afford Cbz-Val-NH_2 as white needles (3.82 g, 90%): mp 200−202 °C (MeCN); $[\alpha]_D$ +23 (*c* 1.0, DMF); IR ν_{max} 3379, 3317, 3294, 1657 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 7.34−7.30 (5H, m), 7.09 (1H, d, J = 9 Hz), 6.99 (1H, br s), 5.02 (2H, ap s), 3.80 (1H, dd, J = 7, 9 Hz), 1.97−1.91 (1H, m), 0.86 (3H, d, J = 7 Hz), 0.82 (3H, d, J = 7 Hz); ^{13}C NMR ($(CD_3)_2SO$) δ 173.1 (s), 156.1 (s), 137.1 (s), 128.2 (d ×4), 127.6 (d), 65.3 (t), 60.0, 30.1, 19.2, 17.9; LRMS (CI, NH_3) *m/z* (rel intensity) 251 ([M + 1]$^+$, 21), 225 (100); HRMS *m/z* calcd for $C_{13}H_{18}N_2O_3$+H 251.1396 (M + H), found 251.1405 (FAB). Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.24; N, 11.19. Found C, 62.50; H, 7.28; N, 10.99.

Cbz-Val-(*Z*)-Aba-Hpp-Hmp-(*R*)-Leu-OBn (15). DCC (203 mg, 0.986 mmol) was added to a solution of **6** (330 mg, 0.931 mmol), **5** (a 4:1 mixture of (*Z*)-:(*E*)-isomers; 454 mg, 0.939 mmol), and DMAP (120 mg, 0.986 mmol) in CH_2Cl_2 (3.1 mL). The reaction mixture was stirred for 1.5 h at 0 °C and then was filtered (to remove the DCU). The combined filtrate and washings was concentrated, and the residue allowed to stand at room temperature overnight. The resulting orange syrup was taken up in a 1:1 mixture of ethyl acetate and hexane and filtered to remove the remaining DCU. The combined filtrate and washings was washed successively with 10% citric acid, saturated $NaHCO_3$(aq), water, and brine and then was dried over Na_2SO_4 and concentrated. The residue was fractionated by FCC (30% ethyl acetate in hexane) to afford **15** as a foam (a 12:1 mixture of (*Z*)-:(*E*)-isomers by HPLC; 687 mg, 91%): $[\alpha]_D$ −9.1 (*c* 1.45, MeOH); IR ν_{max} 3358, 3295, 1731, 1679 cm^{-1}; ^{1}H NMR δ 8.08 (1H, s), 7.35−7.23 (15H, m), 6.97 (1H, q, J = 7 Hz), 6.79 (1H, d, J = 9.5 Hz), 5.55 (1H, d, J = 9 Hz), 5.28−4.95 (6H, m), 4.77 (1H, m), 4.25 (1H, m), 3.31−3.14 (2H, m), 2.12−2.04 (1H, m), 1.81 (3H, d, J = 7 Hz), 1.75−1.45 (5H, m), 1.22−1.17 (1H, m), 0.95−0.70 (18H, m); ^{13}C NMR δ 174.2, 170.0, 169.3, 168.2, 165.8, 156.4, 137.8, 136.4, 135.2, 135.1, 129.6 (×2), 128.8 (×2), 128.7 (×2), 128.6 (×2), 128.5, 128.2, 128.1 (×2), 128.0 (×2), 127.7, 125.3, 74.8, 73.6, 67.6, 67.1, 60.4, 50.3, 41.6, 40.3, 37.4, 31.5, 24.8, 24.3, 23.2, 23.0, 21.9, 21.6, 19.5, 17.9, 15.4; LRMS (FAB) *m/z* (rel intensity) 800 ([M + 1]$^+$, 100); HRMS *m/z* calcd for $C_{45}H_{57}N_3O_{10}$+H 800.4122 (M + H), found 800.4135 (FAB).

Cbz-Val-(*E*)-Aba-Hpp-Hmp-(*R*)-Leu-OBn [(*E*)-15]. Fractionation of **15** (a 10:1 mixture of (*Z*)-:(*E*)-isomers; 30 mg) by PTLC (SiO_2; 90:5:5 benzene, ether, acetone; 5 elutions) gave the more polar **(*Z*)-15** (26 mg, 87%) and **(*E*)-15** (2.5 mg, 8%); IR ν_{max} 3319, 1737, 1676 cm^{-1}; ^{1}H NMR δ 8.16 (1H, s), 7.33−7.15 (16H, m), 6.86 (1H, d, J = 9 Hz), 5.81 (1H, d, J = 9 Hz), 5.42 (1H, dd, J = 4, 7.5 Hz), 5.27−5.01 (5H, m), 4.73 (1H, m), 4.28 (1H, dd, J = 5.5, 8.5 Hz), 3.33 (1H, dd, J = 4, 14.5 Hz), 3.24 (1H, dd, J = 8, 14.5 Hz), 2.25−2.10 (1H, m), 1.97 (3H, d, J = 7.5 Hz), 1.67−1.50 (5H, m), 1.42−1.30 (1H, m), 0.95 (3H, d, J = 6.5 Hz), 0.93−0.87 (9H, m), 0.80 (3H, d, J = 6.5 Hz), 0.79 (3H, d, J = 6.5 Hz); ^{13}C NMR δ 173.9, 170.6, 169.5, 168.3, 166.1, 156.6, 136.5, 135.3, 135.3, 132.3, 129.5 (×2), 129.0 (×2), 128.7 (×2), 128.7 (×2), 128.6, 128.4, 128.2 (×4), 127.7, 124.8, 75.5, 74.4, 67.5, 67.2, 60.8, 50.4, 41.7, 40.4, 37.4, 31.5, 24.9, 24.6, 23.3, 23.1, 21.9, 21.8, 19.4, 17.8, 15.0; LRMS (FAB) *m/z*

(rel intensity) 800 ([M + 1]$^+$, 12), 91 (100); HRMS *m/z* calcd for $C_{45}H_{57}N_3O_{10}$+H 800.4122 (M + H), found 800.4138 (FAB).

Boc-Val-(*Z*)-Aba-Hpp-Hmp-(*R*)-Leu (17). 10% Pd/C (0.572 g) was added under an argon atmosphere to a degassed solution of **15** (0.286 g, 0.36 mmol) and $(Boc)_2O$ (0.118 g, 0.54 mmol) in absolute ethanol (2.4 mL), followed by the slow addition of 1,4-cyclohexadiene (0.34 mL, 3.6 mmol). After 4 h of stirring at room temperature, the catalyst was filtered off, and the combined filtrate and washings was concentrated. The residue was fractionated by MPC (SiO_2, 7.5 g; CH_2Cl_2/MeOH/AcOH, 95:5:0.2) to provide **17** as a white foam (225 mg, 92%): $[\alpha]_D$ −20 (*c* 1.4, MeOH); IR ν_{max} 3378, 3291, 1720, 1674 cm^{-1}; ^{1}H NMR δ {major rotamer} 8.15 (1H, s), 7.31−7.25 (5H, m), 6.83 (1H, q, *J* = 7 Hz), 6.77 (1H, d, *J* = 9.5 Hz), 5.35−5.21 (2H, m), 4.70 (1H, m), 4.23 (1H, dd, *J* = 6.5, 9.5 Hz), 3.30 (1H, dd, *J* = 4, 14.5 Hz), 3.20 (1H, dd, *J* = 7.5, 14.5 Hz), 2.04 (1H, m), 1.81 (3H, d, *J* = 7 Hz), 1.72−1.20 (7H, m), 1.42 (9H, s), 0.94 (12H, m), 0.78 (6H, m); ^{13}C NMR δ 175.7, 170.0, 169.2, 168.5, 165.9, 156.9, 136.2, 135.4, 129.6 (×2), 128.8 (×2), 127.5, 125.2, 81.0, 75.2, 73.5, 59.9, 50.2, 42.0, 40.2, 37.5, 31.9, 28.5 (×3), 24.9, 24.3, 23.3, 23.0, 22.0, 21.6, 19.5, 17.8, 15.8; LRMS (FAB) *m/z* (rel intensity) 676 ([M + 1]$^+$, 8), 72 (100); HRMS *m/z* calcd for $C_{35}H_{53}N_3O_{10}$+H 676.3809 (M + H), found 676.3800 (FAB).

Boc-Val-(*Z*)-Aba-Hpp-Hmp-(*R*)-Leu-OPfp (18). DCC (52 mg, 0.25 mmol) was added to a solution of **17** (171 mg, 0.253 mmol) and C_6F_5OH (47 mg, 0.25 mmol) in dry ethyl acetate (2.5 mL) at 0 °C under argon. After the reaction mixture was stirred for 2.5 h at 0 °C, the DCU was filtered off, the combined filtrate and washings was concentrated, and the residue was fractionated by MPC (SiO_2, 7.5 g; 25% ethyl acetate in hexane) to give **18** as a white foam (179 mg, 84%): $[\alpha]_D$ −0.10 (*c* 1.8, $CHCl_3$); IR ν_{max} 3298, 3287, 1791, 1722, 1671 cm^{-1}; ^{1}H NMR δ 7.54 (1H, s), 7.34−7.22 (5H, m), 6.99 (1H, d, *J* = 8 Hz), 6.88 (1H, q, *J* = 7 Hz), 5.27 (2H, m), 5.02 (2H, m), 3.76 (1H, dd, *J* = 7.5, 8.5 Hz), 3.28 (1H, dd, *J* = 4, 14.5 Hz), 3.21 (1H, dd, *J* = 7, 14.5 Hz), 1.98 (1H, m), 1.80−1.15 (6H, m), 1.78 (3H, d, *J* = 7 Hz), 1.39 (9H, s), 0.97 (6H, d, *J* = 5 Hz), 0.91 (3H, d, *J* = 7 Hz), 0.88 (3H, d, *J* = 7 Hz), 0.76 (3H, d, *J* = 6.5 Hz), 0.74 (3H, d, *J* = 6.5 Hz); ^{13}C NMR δ 170.7, 170.5, 170.0, 168.4, 165.7, 155.8, 137.3, 135.3, 129.7 (×2), 128.9 (×2), 127.6, 125.7, 80.0, 74.8, 73.8, 60.1, 50.1, 41.0, 40.3, 37.4, 31.0, 28.4 (×3), 24.9, 24.3, 23.2, 23.1, 21.7 (×2), 19.3, 18.1, 15.0; LRMS (FAB) *m/z* (rel intensity) 842 ([M + 1]$^+$, 9), 72 (100); HRMS *m/z* calcd for $C_{41}H_{52}F_5N_3O_{10}$: 841.3573, found 841.3577 (EI).

Cyclo[(2*R*, 3*R*)-3-(PhSe)Abu-Hpp-Hmp-(*R*)-Leu-Val] (19a). BuLi (1 M, 0.011 mL, 0.011 mmol) was added to a solution of **2** (129 mg, 0.231 mmol) and benzeneselenol (0.073 mL, 0.694 mmol) in THF (0.5 mL) at 0 °C under argon, and the mixture was heated under reflux for 24 h. The cooled (rt) reaction mixture was diluted with water and extracted with ethyl acetate, and the combined organic layers was washed with water, dried over Na_2SO_4, concentrated, and fractionated by FCC (40% ethyl acetate in hexane) to give **19c** (oil, 10 mg, 6%) [^{1}H NMR δ 7.62−7.11 (10H, m), 6.83 (1H, d, *J* = 9 Hz), 6.29 (1H, d, *J* = 9 Hz), 6.11 (1H, d, *J* = 10 Hz), 5.36 (1H, dd, *J* = 4.5, 9 Hz), 4.89 (1H, dd, *J* = 5, 9 Hz), 4.71 (1H, dd, *J* = 4, 7.5 Hz), 4.44 (1H, ddd, *J* = 7.5, 7.5, 8 Hz), 4.07 (1H, dd, *J* = 10, 10 Hz), 3.46 (1H, dq, *J* = 5, 7 Hz), 3.01 (1H, dd, *J* = 8, 14.5 Hz), 2.92 (1H, dd, *J* = 4, 14.5 Hz), 2.13 (1H, m), 1.9−1.4 (6H, m), 1.29 (3H, d, *J* = 7 Hz), 0.98−0.89 (12H, m), 0.79 (3H, d, *J* = 7 Hz), 0.76 (3H, d, *J* = 6.5 Hz)], **19b** (oil, 19 mg, 12%) [^{1}H NMR (C_6D_6) δ 7.84 (2H, ap d, *J* = 7 Hz), 7.31−7.0 (10H, m), 6.55 (1H, d, *J* = 9 Hz), 5.42−5.28 (2H, m), 5.09 (1H, dd, *J* = 6, 6.5 Hz), 4.75 (1H, dd, *J* = 5.5, 9.5 Hz), 4.28 (1H, m), 4.02 (1H, m), 3.07 (1H, dd, *J* = 7, 14 Hz), 2.97 (1H, dd, *J* = 5, 14 Hz), 2.38 (1H, m), 1.78−1.51 (6H, m, *J* = 7 Hz), 1.70 (3H, d), 0.97−0.88 (12H, m), 0.84 (6H, d, *J* = 6.5 Hz)], and **19a** (85 mg, 52%) [$[\alpha]_D$ −56 (*c* 1.3, $CHCl_3$); IR ν_{max} 3269, 1744, 1671, 1655 cm^{-1}; ^{1}H NMR δ 7.63−7.60 (2H, m), 7.38−7.22 (9H, m), 6.97 (1H, d, *J* = 9 Hz), 6.40 (1H, d, *J* = 9 Hz), 5.32 (1H, dd, *J* = 6.5, 7.5 Hz), 5.19 (1H, dd, *J* = 7, 7 Hz), 4.83 (1H, dd, *J* = 7.5, 8.5 Hz), 4.38 (1H, dd, *J* = 4.5, 9 Hz), 4.30 (1H, ddd, *J* = 7.5, 7.5, 7.5 Hz), 3.64 (1H, dq, *J* = 7, 7 Hz), 3.21 (1H, dd, *J* = 6.5, 14.5 Hz), 3.16 (1H, dd, *J* = 7.5, 14.5 Hz), 2.40 (1H, m), 1.69 (2H, m), 1.56 (4H, m), 1.31 (3H, d, *J* = 7.0 Hz), 0.96−0.90 (18H, m); ^{13}C NMR δ 171.7, 171.0, 170.8, 168.8, 168.3, 135.2 (×2), 135.0, 129.6 (×2), 129.3 (×2), 128.8 (×2), 128.7, 128.1, 127.5, 77.4, 75.5, 73.5, 59.6, 58.0, 53.2, 40.1, 39.2, 38.9, 36.6, 29.8, 25.0, 24.7, 22.3, 22.6, 22.5 (×2), 19.9, 19.6, 17.6; LRMS (FAB) *m/z* (rel intensity) 715 ([M]$^+$, 100); HRMS *m/z* calcd for $C_{36}H_{49}N_3O_7Se$ 715.2736, found 715.2734 (FAB)].

(*R*)-5-Ethylidene-2-(2-methyl-1-(benzyloxycarbonylamido)propyl)oxazol-4(5*H*)-one (20). EDC (59 mg, 0.31 mmol) was added at once to a suspension of the acid **5** (*Z*):(*E*), 4:1; 103 mg, 0.31 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C. After 30 min of stirring, the reaction mixture was diluted with ethyl acetate and hexane (1:1) and washed with H_2O (×3), dried over Na_2SO_4, and concentrated to give **20** as a 5:1 mixture of (*Z*)-:(*E*)-isomers (87 mg, 90%): IR ν_{max} 3321, 1805, 1726, 1681 cm^{-1}; ^{1}H NMR δ {(*Z*)-isomer} 7.25−7.35 (5H, m), 6.68 (1H, q, *J* = 7.5 Hz), 5.40 (1H, d, *J* = 9 Hz), 5.07−5.16 (2H, m), 4.63 (1H, dd, *J* = 5.5, 9 Hz), 2.20 (1H, m), 2.13 (3H, d, *J* = 7.5 Hz), 1.00 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 7 Hz); {(*E*)-isomer} 6.82 (1H, q, *J* = 8 Hz), 2.27 (3H, d, *J* = 8 Hz); ^{13}C NMR δ {(*Z*)-isomer} 166.3, 165.4, 156.2, 136.3, 136.2, 135.6, 128.7 (×2), 128.4, 128.3 (×2), 67.4, 55.4, 31.4, 19.1, 17.6, 14.8; LRMS (FAB) *m/z* (rel intensity) 317 ([M + 1]$^+$, 47), 90 (100); HRMS *m/z* calcd for $C_{17}H_{20}N_2O_4$+H 317.1501 (M + H), found 317.1509 (FAB).

Cbz-Val-Aba-Hpp-OMe (22). DCC (62 mg, 0.30 mmol) was added to a stirred solution of **5** ((*Z*):(*E*) 4:1; 100 mg, 0.30 mmol), **21** (54 mg, 0.30 mmol), and DMAP (67 mg, 0.30 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. After 1 h, the reaction mixture was concentrated, and after 18 h of standing at room temperature, the residue was taken up in 1:1 (v/v) mixture of ethyl acetate and hexane and filtered. The combined filtrate and washings was concentrated and fractionated by FCC (SiO_2; 35% ethyl acetate in hexane) to provide recovered **21** (8 mg, 15%), (*Z*)-**22** (88 mg, 59%), and (*E*)-**22** (20 mg, 13%). For (*Z*)-**22**: IR ν_{max} 3292, 1745, 1691, 1668 cm^{-1}; ^{1}H NMR δ 7.36−7.18 (11H, m), 6.87 (1H, q, *J* = 7 Hz), 5.44 (1H, d, *J* = 8.5 Hz), 5.27 (1H, dd, *J* = 6, 6.5 Hz), 5.09 (2H, s), 4.18 (1H, dd, *J* = 6, 8.5 Hz), 3.67 (3H, s), 3.23−3.10 (2H, m), 2.21−2.04 (1H, m), 1.74 (3H, d, *J* = 7 Hz), 0.99 (3H, d, *J* = 7 Hz), 0.93 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 170.0, 169.8, 163.7, 156.7, 136.4 (×2), 135.8, 129.5 (×2), 128.8 (×2), 128.7 (×2), 128.4, 128.3 (×2), 127.4, 125.4, 73.9, 67.4, 60.6, 52.6, 37.6, 31.4, 19.5, 17.9, 15.1; LRMS (EI) *m/z* (rel intensity) 496 ([M]$^+$, 3), 91 (100); HRMS *m/z* calcd for $C_{27}H_{32}N_2O_7$ 496.2210, found 496.2205 (EI). For (*E*)-**22**: IR ν_{max} 3294, 1752, 1728, 1687, 1660 cm^{-1}; ^{1}H NMR δ 7.56 (1H, s), 7.34−7.18 (11H, m), 5.40 (1H, dd, *J* = 5, 7.5 Hz), 5.28 (1H, d, *J* = 8.5 Hz), 5.11 (2H, s), 4.00 (1H, m), 3.74 (3H, s), 3.26 (1H, dd, *J* = 4.5, 14.5 Hz), 3.18 (1H, dd, *J* = 8, 14.5 Hz), 2.11 (1H, m), 2.04 (3H, d, *J* = 7.5 Hz), 0.94 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 7.0 Hz); ^{13}C NMR δ 170.0 (s), 169.7 (s), 163.4 (s), 156.5 (s), 136.4 (s), 135.6 (s), 131.0 (d), 129.4 (d × 2), 128.9 (d × 2), 128.8 (d × 2), 128.4 (d), 128.3 (d × 2), 127.5 (d), 124.7 (s), 74.1 (d), 67.4 (t), 61.3 (d), 52.8 (q), 37.5 (t), 31.3 (d), 19.4 (q), 17.8 (q), 14.7 (q); LRMS (EI) *m/z* (rel intensity) 496 ([M]$^+$, 2), 91 (100); HRMS *m/z* calcd for $C_{27}H_{32}N_2O_7$ 496.2210, found 496.2211 (EI).

Boc-Val-OPfp (24). DCC (867 mg, 4.2 mmol) was added to a stirred solution of Boc-Val (869 mg, 4.0 mmol) and C_6F_5-OH (736 mg, 4.0 mmol) in ethyl acetate (4.0 mL) at 0 °C. After 1.5 h, the DCU was removed by filtration, and the combined filtrate and washings was concentrated and fractionated by FCC (SiO_2; 15% ethyl acetate in hexane) to provide **24** (1.49 g, 97%) as a white solid: $[\alpha]_D$ −18.1 (*c* 1.0, $CHCl_3$); IR ν_{max} 3294, 1786, 1712, 997 cm^{-1}; ^{1}H NMR δ 5.05 (1H, d, *J* = 9 Hz), 4.55 (1H, dd, *J* = 5, 9 Hz), 2.35−2.29 (1H, m), 1.45 (9H, s), 1.07 (3H, d, *J* = 7 Hz), 1.01 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 168.9, 155.6, 141.4 (dm ×2, J_{CF} = 252 Hz), 139.9 (dm, J_{CF} = 254 Hz), 138.1 (dm ×2, J_{CF} = 249 Hz), 125.0 (br s), 80.6, 58.9, 31.3, 28.4, 19.1, 17.6; LRMS (Cl) *m/z* (rel intensity) 401 ([M + 18]$^+$, 14), 345 (100); HRMS *m/z* calcd for $C_{16}H_{18}F_5NO_4$+NH_4 401.1500 (M + NH_4), found 401.1501 (CI, NH_3). Anal. Calcd for $C_{16}H_{18}F_5NO_4$: C, 50.14; H, 4.37; N, 3.65. Found C, 50.20; H, 4.50; N, 3.91.

Boc-Val-(*R*)-Abu [(*R*)-25]. Boc-Val-OPfp (**24**; 380 mg, 1.0

mmol) was added at once to a stirred solution of (2*R*)-2-aminobutanoic acid (103 mg, 1.0 mmol) and Na_2CO_3 (106 mg, 1.0 mmol) in *t*-BuOH (2.5 mL) and H_2O (2.5 mL) at room temperature. After 13 h, the mixture was acidified (pH = ca. 3) by addition of citric acid and then was extracted with ethyl acetate (×3). The combined organic layers was washed with brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (SiO_2; CH_2Cl_2/CH_3OH/AcOH, 95:5:0.2) to provide (*R*)-**25** as a white solid (226 mg, 75%): $[\alpha]_D$ −10.9 (*c* 1.0, MeOH); IR ν_{max} 3310, 1720, 1649 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 8.02 (1H, d, J = 7.5 Hz), 6.59 (1H, d, J = 9 Hz), 4.12 (1H, ddd, J = 5, 8, 8 Hz), 3.87 (1H, dd, J = 7, 8.5 Hz), 1.95−1.87 (1H, m), 1.77−1.55 (2H, m), 1.38 (9H, s), 0.88−0.80 (9H, m); ^{13}C NMR δ 174.5, 171.9, 156.7, 80.7, 59.6, 53.5, 32.0, 28.4 (×3), 25.5, 19.4, 19.2, 9.4; LRMS (Cl) *m*/*z* (rel intensity) 303 ($[M + 1]^+$, 35), 72 (100); HRMS *m*/*z* calcd for $C_{14}H_{26}N_2O_5$+H 303.1920 (M + H), found 303.1927 (CI, NH_3). Anal. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.61; H, 8.67; N, 9.26. Found C, 55.70; H, 8.50; N, 9.31.

Boc-Val-(*S*)-Abu [(*S*)-25]. Reaction of (2*S*)-2-aminobutanoic acid (103 mg, 1.0 mmol) with **24** (383 mg, 1.0 mmol) as described above gave, after fractionation by FCC (SiO_2; 10% CH_3OH in CH_2Cl_2), (*R*)-**25** as a foam (211 mg, 70%): mp 174−175 °C (MeOH/ether); $[\alpha]_D$ −33 (*c* 1.0, MeOH); IR ν_{max} 3316, 1720, 1690, 1649 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 7.88 (1H, d, J = 7.5 Hz), 6.72 (1H, d, J = 9 Hz), 4.15 (1H, ddd, J = 5, 7.5, 7.5 Hz), 3.81 (1H, dd, J = 7, 9 Hz), 2.05−1.85 (1H, m), 1.80−1.50 (2H, m), 1.38 (9H, s), 0.85 (3H, t, J = 7 Hz), 0.84 (3H, d, J = 7 Hz), 0.81 (3H, d, J = 7 Hz); ^{13}C NMR δ 175.7 (s), 172.8 (s), 156.6 (s), 80.5 (s), 60.4 (d), 53.6 (d), 30.9 (d), 28.4 (q ×3), 25.2 (t), 19.3 (q), 18.4 (q), 9.7 (q); LRMS (Cl) *m*/*z* (rel intensity) 303 ($[M + 1]^+$, 84), 203 (100); HRMS *m*/*z* calcd for $C_{14}H_{26}N_2O_5$+H 303.1920 (M + H), found 303.1920 (CI, NH_3).

(*R*)-Hpp-Hmp-(*R*)-Leu-OBn (26). Oxalyl chloride (0.244 mL, 2.8 mmol) was added to a stirred solution of *ent*-**11** (947 mg, 2.4 mmol) and DMF (0.018 mL, 0.24 mmol) in CH_2Cl_2 (4.0 mL) at 0 °C under argon. After 30 min of stirring at 0 °C and 4 h of stirring at room temperature, the mixture was diluted with hexane (4.0 mL) and filtered, and the combined filtrate and washings was concentrated. To the resulting residue was added a solution of **10** (671 mg, 2.0 mmol) and DMAP (293 mg, 2.4 mmol) in CH_2Cl_2 (4.0 mL) at 0 °C. After 15 min, the mixture was allowed to warm to room temperature and stirred at that temperature for 2 h. The reaction mixture was diluted with ethyl acetate and hexane (1:1) and was washed sequentially with water, 10% citric acid, saturated $NaHCO_3$(aq), and brine, dried over Na_2SO_4, and concentrated. The residue was taken up in 5% HF/CH_3CN (20 mL), and after 2.5 h, the mixture was diluted with ethyl acetate and hexane (1:1) and was washed sequentially with water, saturated $NaHCO_3$(aq), and brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (SiO_2, 30% ethyl acetate in hexane) to afford **26** as a white solid (873 mg, 90%): mp 100−101 °C ($CHCl_3$/hexane); $[\alpha]_D$ −9.4 (*c* 1.0, $CHCl_3$); IR ν_{max} 3423, 3326, 1750, 1731 cm^{-1}; ^1H NMR δ 7.39−7.22 (10H, m), 6.23 (1H, d, J = 8 Hz), 5.21 (1H, dd, J = 4, 8.5 Hz), 5.16 (2H, m), 4.66−4.59 (1H, m), 4.50 (1H, m), 3.16 (1H, dd, J = 5, 14 Hz), 2.98 (1H, dd, J = 7.5, 14 Hz), 2.65 (1H, br s), 1.74−1.45 (6H, m), 0.91 (12H, m); ^{13}C NMR δ 173.5, 172.6, 169.7, 136.4, 135.5, 129.6, 128.8, 128.8, 128.5, 127.2, 73.9, 71.8, 67.4, 50.8, 41.5, 40.8, 40.6, 25.1, 24.6, 23.3, 23.0, 22.1, 21.8; LRMS (FAB) *m*/*z* (rel intensity) 484 ($[M + 1]^+$, 38), 91 (100); HRMS *m*/*z* calcd for $C_{28}H_{37}NO_6$+H 484.2699 (M + H). Found 484.2701 (FAB). Anal. Calcd for $C_{28}H_{37}NO_6$: C, 69.54; H, 7.71; N, 2.90. Found C, 60.71; H, 7.62; N, 3.11.

Boc-Val-(*R*)-Abu-Hpp-Hmp-(*R*)-Leu-OBn [(*R*)-27]. Diethyl azodicarboxylate (0.031 mL, 0.20 mmol) was added dropwise via syringe over 2 min to a stirred solution of acid (*R*)-**25** (60 mg, 0.20 mmol), alcohol **26** (91 mg, 0.19 mmol), and Ph_3P (52 mg, 0.20 mmol) in THF (0.9 mL) at 0 °C under argon. After 19 h of stirring at room temperature, the mixture was concentrated, and the residue was fractionated by FCC (SiO_2; 30% ethyl acetate in hexane) to provide (*R*)-**27** as a white foam (128 mg, 89%): $[\alpha]_D$ −44 (*c* 1.0, $CHCl_3$); IR ν_{max} 3363, 3317, 1745, 1718, 1660 cm^{-1}; ^1H NMR δ 7.41−7.22 (10H, m), 6.84 (1H, d, J = 7 Hz), 6.63 (1H, d, J = 9 Hz), 5.58 (1H, d, J = 8.5 Hz), 5.23−5.12 (4H, m), 4.71 (1H, m), 4.63 (1H, m), 4.12 (1H, m), 3.30 (1H, dd, J = 3.5, 14.5 Hz), 3.15 (1H, dd, J = 8.5. 14.5 Hz), 2.30−2.10 (1H, m), 1.90−1.72 (1H, m), 1.72−1.20 (7H, m), 1.41 (9H, s), 0.98−0.87 (9H, m), 0.86−0.68 (12H, m); ^{13}C NMR δ 173.9, 173.7, 171.6, 169.7, 168.2, 156.2, 135.4, 135.3, 129.6 (×2), 128.9 (×2), 128.8 (×2), 128.6, 128.3 (×2), 127.6, 79.9, 74.7, 74.3, 67.5, 59.7, 53.5, 50.4, 41.6, 40.3, 37.3, 30.6, 28.5 (×3), 25.6, 24.9, 24.5, 23.2 (×2), 21.7, 21.7, 19.6, 17.6, 9.3; LRMS (EI) *m*/*z* (rel intensity) 767 ($[M]^+$, 2), 72 (100); HRMS *m*/*z* calcd for $C_{42}H_{61}N_3O_{10}$ 767.4357, found 767.4359 (EI).

Boc-Val-(S)-Abu-Hpp-Hmp-(R)-Leu-OBn [(*S*)-27]. Mitsunobu reaction of (*S*)-**25** (100 mg, 0.331 mmol) with **26** (168 mg, 0.348 mmol) as described above gave, after fractionation by FCC (SiO_2; 30% ethyl acetate in hexane), recovered **26** (28 mg, 17%) and (*S*)-**27** as a foam (159 mg, 63%): $[\alpha]_D$ −38 (*c* 1.5, $CHCl_3$); IR ν_{max} 3373, 3317, 1745, 1658 cm^{-1}; ^1H NMR δ 7.40−7.20 (10H, m), 7.05 (1H, d, J = 8 Hz), 6.72 (1H, d, J = 9.5 Hz), 5.30−5.19 (5H, m), 4.82−4.68 (2H, m), 4.06 (1H, dd, J = 7.5, 8 Hz), 3.27 (1H, dd, J = 4, 14.5 Hz), 3.19 (1H, dd, J = 6.5, 14.5 Hz), 2.01−1.85 (2H, m), 1.85−1.68 (1H, m), 1.68−1.52 (5H, m), 1.52−1.38 (1H, m), 1.42 (9H, s), 0.96−0.86 (15H, m), 0.76 (3H, d, J = 6.5 Hz), 0.74 (3H, d, J = 6.5 Hz); ^{13}C NMR δ 174.5, 173.9, 172.0, 169.4, 168.0, 155.9, 135.3, 135.0, 129.9 (×2), 128.8 (×4), 128.6, 128.3 (×2), 127.6, 79.8, 74.9, 73.5, 67.6, 59.8, 53.3, 50.4, 41.6, 40.7, 37.3, 31.6, 28.5 (×3), 25.7, 24.9, 24.0, 23.3, 23.1, 21.9, 21.4, 19.5, 18.0, 10.1; LRMS (EI) *m*/*z* (rel intensity) 767 ($[M]^+$, 1), 121 (100); HRMS *m*/*z* calcd for $C_{42}H_{61}N_3O_{10}$+H 768.4435 (M + H), found 768.4436 (FAB).

Phytotoxicity Bioassays. Canola, cv. Westar (susceptible to virulent *P. lingam* isolates), brown mustard cv. Cutlass (resistant to virulent *P. lingam* isolates), and white mustard cv. Ochre (resistant to virulent *P. lingam* isolates) plants were grown under controlled environmental conditions (controlled environment chamber at 20/18 °C with a 16/8 h day/night cycle). Leaves of 21-day-old plants were punctured with a needle, and a 10-μL droplet of the test solution dissolved in 50% aqueous (v/v) methanol was placed on each wound. The symptoms that developed within 2−5 days varied from little or no reaction to brownish-yellow lesions. In no case were lesions observed with 50% aqueous (v/v) methanol.

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